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PHYSIOLOGICAL STUDIES OF SEVERAL PATHOGENIC BACTERIA THAT INDUCE CELL STIMULATION IN PLANTS [1]

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INTRODUCTION

The nature of the stimuli responsible for atypical and pathological growth of living cells is of extensive biological interest. A group of closely related diseases caused by bacteria which induce such growths in higher plants seems to offer favorable material for studies in this field. Of these diseases, crown gall has received the most attention. It was first studied intensively by E. F. Smith and his associates, who considered it to be similar in many respects to certain types of sarcomata and who referred to it as plant cancer. Riker and Berge (*15*) [3] have made a critical review of the literature on crown gall and have pointed out certain advantages offered by plant material for fundamental studies on the nature and cause of such growths. They have suggested methods of testing the hypotheses put forward by workers investigating the cause and nature of stimuli concerned with atypical and pathological growths. The present paper, an abstract of which has already been published (*11*), and which reports the results of a unit in a larger Wisconsin project, is concerned with various aspects of the comparative physiology of a group of cell-stimulating plant pathogens.

The plant pathogens examined in this study were, for the most part, well known. They were *Phytomonas tumefaciens* (Smith and Town.) Bergey et al., which causes crown gall of plants; *P. beticola* (Smith, Brown, and Town.) Bergey et al., the causal agent of bacterial pocket rot of sugar beets which is designated as beet gall in this paper; *P. savastanoi* (E. F. Smith) Bergey et al., var. *nerii* C. O. Smith, which causes oleander tubercle or gall; *P. savastanoi* (E. F. Smith) Bergey et al., the causal agent of olive knot; and raspberry cane gall, an unnamed bacterium producing overgrowths on the canes of black raspberry plants.

The diseases caused by these pathogens are characterized by large overgrowths composed of hypertrophic and hyperplastic tissues often containing disorganized vascular elements and sometimes conspicuous pockets of necrotic tissue. Elcock (*6*) elaborated on the intercellular

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[3] Reference is made by number (italic) to Literature Cited, p. 951.

position of the bacteria in galls of sugar beets earlier mentioned by E. F. Smith et al. (*20*). Similarly, C. O. Smith (*18*), working on oleander tubercle, found the same bacterial position in connection with this disease. E. F. Smith (*19*) had studied the olive knot disease and found the bacteria in the intercellular spaces of the host, together with abnormal tissue development and bacterial cavities. Recent unpublished work by W. M. Banfield on the raspberry cane gall disease showed this malady to be closely related to the others with respect to cellular stimulation of the host and the position of the bacteria in the intercellular spaces. Since the bacteria causing crown gall (*12*, *13*, *16*) were found primarily in the intercellular spaces of the host, it appeared that this disease was correspondingly similar to the others and that one of the chief differences, the formation of necrotic pockets, was mostly one of degree.

After a consideration of these morphological similarities of the host-parasite relations, and of the pathogenicity of the various cultures, a comparative study of certain physiological characteristics of the causal organisms was undertaken.

IDENTIFICATION OF CULTURES EMPLOYED

The cultures of bacteria employed in this study were obtained through the courtesy of several investigators as follows: (1) The crown gall organism, *Phytomonas tumefaciens*, came from A. J. Riker. Its earlier history (*14*) was given under the designation A–1. (2) The cane gall organism was secured from Banfield (*3*) who isolated it from a gall on a black raspberry stem grown in northern Indiana. So far as the writer is aware, this organism has not been named. (3) The beet gall organism, *P. beticola*, was obtained from A. J. Quirk. This culture was a "rough type" as described by Brown (*4*). (4) The oleander organism, *P. savastanoi* var. *nerii*, was secured from diseased material collected in California by C. O. Smith. (5) The olive knot organism, *P. savastanoi*, was obtained from diseased material collected in California, by the H. J. Heinz Corporation.

Isolations from the diseased materials were made in the usual way, and the pathogenicity of all of the cultures established before further purification was undertaken. The details of this work are omitted because of their volume.

Single-cell cultures were made according to the methods of Wright et al. (*23*), with slight modifications suggested by A. A. Hendrickson. Yeast-extract-manitol-mineral-salts liquid medium (described later) was used. The parent cultures were incubated 18 to 20 hours at 24° C. before isolations were attempted. Microdrops from this culture were made beside large drops of sterile media on sterile cover slips until an individual cell appeared. If more than one cell was placed in the microdrop, the cover slip was discarded. The microdrop was incubated for 24 to 48 hours before it was mixed with the adjacent large drop.

The pathogenicity of the single-cell cultures was determined through a series of inoculations on suitable hosts. The details of this work also are omitted because of their volume.

CROSS INOCULATIONS

Cross inoculations were made with these cultures to determine their pathogenicity in the plants used. The ability of each organism

to parasitize the hosts listed in table 1 was tested (1) with cultures from poured-plate isolations, (2) with progenies of single-cell cultures, and (3) with bits of gall tissue from each host. Since the results with these different methods were similar, they are considered together. The inoculations were made and repeated during the winters of 3 consecutive years on plants grown in the greenhouse at approximately 24° C. Only vigorously growing organisms and plant materials were used. The incubation period ranged from a minimum of 5 days for the beet gall organism on garden beets to a maximum of 20 days for the olive knot organism on olives. Failure to produce infection on a suitable host under the above-described conditions was rarely encountered even with organisms kept over 3 years in culture.

TABLE 1.—*Results of inoculations with various gall-forming bacteria on several plants*

Organisms with which inoculations were made	Pathogenicity [1] on—				
	Bonny Best tomato	Cumberland black raspberry	Detroit dark red beet	Pink and white oleander	Manzanillo olive
Crown gall	+	+	+	+	+
Cane gall	0	+	0	0	0
Beet gall	0	0	+	0	0
Oleander gall	0	0	0	+	+
Olive knot	0	0	0	[2] 0	+

[1] Positive results indicated by +; negative results by 0. During the course of this work over 100 inoculations with the organism on its proper host and over 20 inoculations of each organism on each host were made.

[2] One of the twenty inoculations suggested positive results.

The results of these inoculation studies are summarized in table 1. No differences worthy of note were observed either in different trials or with the various sources of inoculum. Portions of this work confirm that of E. F. Smith (*19*) and C. O. Smith (*18*). The details are omitted because of their volume.

PRELIMINARY PHYSIOLOGICAL STUDIES

A further means of identification was obtained by growing the various pathogens on the substrata mentioned in the literature, which provided characteristic reactions.

The results of these studies are summarized in table 2. As far as this work was carried, the results agreed closely with those of Brown (*4*), Riker (*14*), C. O. Smith (*18*), and E. F. Smith (*19*). It was also suggested that the undescribed cane gall organisms might be distinct from all the others because (1) it was very slow growing in all the media, and (2) it produced a different type of growth in litmus milk, characterized by a slight serum zone, pink color, acid reaction, and curd after an incubation period of 3 weeks at 24° C. Further differences appeared during the investigations on carbon and nitrogen utilization mentioned later. None of these media showed clear differentiation between the bacteria causing oleander gall and olive knot. C. O. Smith (*18*) concluded that these two organisms were almost identical as far as his cultural studies were carried.

TABLE 2.—*Summary of results obtained with cultures of various gall-forming bacteria on different media*

Test and media	Results with organism causing—				
	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot
Growth[1] on (in)—					
Nutrient glucose agar	++	+	+++	+++	+++
Nutrient glucose broth	++	+	+++	+++	+++
Nutrient agar	++	+	+++	+++	+++
Yeast-extract-glucose agar	++	++	+++	+++	+++
Yeast-extract-glucose liquid medium	+++	+++	+++	+++	+++
Potato-glucose agar	+++	++	+++	+++	+++
Litmus milk	++	++	++	++	++
Serum zone	Present	Present	Present	None	None
Color	Gray to brown.	Pink	White to brown.	Blue	Blue
Reaction	Alkaline	Acid	Acid	Alkaline	Alkaline
Liquefaction of gelatin[2]	0	0	+	0	0
Hydrolysis of starch[2]	0	0	+	0	0
Digestion of casein[2]	0	0	+	0	0

[1] Amount of growth indicated as follows: +, slight; ++, moderate; +++, abundant.
[2] Results indicated as follows: 0, negative; +, positive.

COMPARATIVE PHYSIOLOGICAL STUDIES

METHODS AND APPARATUS

The bacteriological work reported in this paper was performed in a special room with the temperature controlled at approximately 24° C. The air of the room was supplied through oiled-paper air filters which kept dust and contamination at a minimum. The methods of the Society of American Bacteriologists (*21*) were employed unless otherwise noted. The bacterial suspensions used in seeding the media were prepared according to a standardized technic as follows: The density of a water suspension from a 2-day-old potato-glucose agar culture was adjusted to approximately 1.0 of the McFarland (*8*) scale. The cultures were seeded in duplicate with one drop of suspension and allowed to incubate 21 days at the temperature of the room. Experiments were repeated at least three times unless otherwise noted. Special technics are described under appropriate headings.

The selection of a synthetic basic medium which contained suitable sources of carbon and nitrogen readily available to all of the cultures and which would support growth without releasing objectionable end products was desired. Yeast-extract-glucose-mineral-salts medium, while not possessing all the above requirements, proved satisfactory. Its composition was as follows: 1-percent yeast extract, 100 cc; glucose, 5 g; magnesium sulphate ($MgSO_4.7H_2O$), 0.2 g; calcium chloride, ($CaCl_2$), 0.1 g; sodium chloride (NaCl), 0.2 g; dipotassium phosphate ($K_2HPO_4.3H_2O$), 0.2 g; and water, 900 cc. The reaction was adjusted to approximately pH 7.0. The yeast extract was made according to the method of Allyn and Baldwin (*2*). Tests for the presence of reducing sugars in the yeast extract both before and after hydrolysis were negative. However, a trace of growth was made by the bacteria, as may be seen later when the glucose was omitted. Attempts to prevent this growth by fermentation of the yeast infusion with *Bacillus coli communis* were unsuccessful.

Hydrogen-ion concentrations were measured potentiometrically by means of a glass electrode together with a vacuum-tube circuit slightly modified from that described by Partridge (*10*). A diagrammatic representation of the equipment together with the electrode assembly is given in figure 1.

The glass electrodes or half cells were made essentially by the procedure of MacInnes and Dole (*9*).

Calibration was effected by determining the electrode potentials of various standard buffers covering the pH range of the experiments studied. The error in the measurements, between pH 1.5 and 11.0, was found to be less than ± 0.02 pH unit for any single

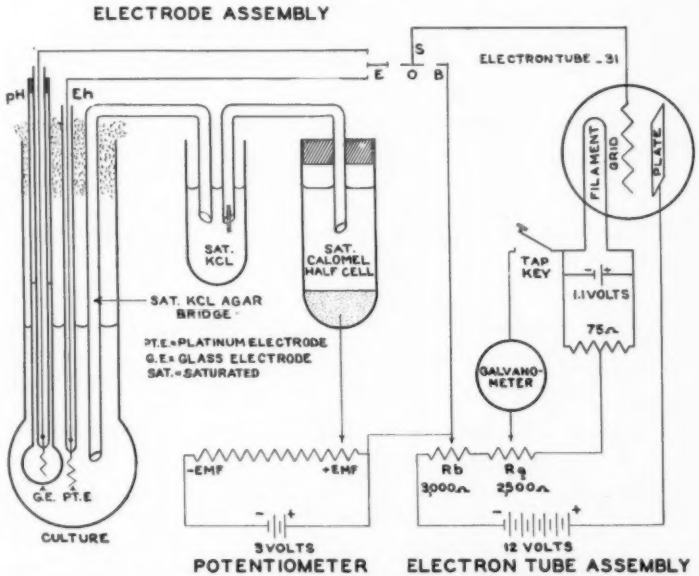

FIGURE 1.—Diagram of vacuum-tube galvanometer and electrode assembly for measuring oxidation-reduction intensities and hydrogen-ion concentrations with the platinum and glass electrodes, respectively. With switch *S* in the *O* position, the variable resistance *Rg* is adjusted with the aid of the tap key until the galvanometer reads zero. Switch *S* is then placed in the *B* position and the variable resistance *Rb* is adjusted until the galvanometer again reaches zero. Both these positions of switch *S* are checked again. When they are balanced at zero, switch *S* is then moved to the *E* position. The electrode potentials of the platinum or glass are read from the potentiometer in the usual manner and the values interpolated to E_h or pH, respectively.

experiment. These limits of error were determined by calibration before and after each experiment. All measurements were recorded to the nearest 0.1 pH unit.

Oxidation-reduction intensities were measured potentiometrically by substituting two bright platinum electrodes for the glass half cell; no other modifications of the apparatus were necessary. The operation of similar apparatus has been described by Allyn and Baldwin (*2*) and by others.

The yeast-extract-glucose liquid medium as employed in these trials showed little change in oxidation-reduction intensity whether it

was open to, or sealed from, the air. Figure 2 (control) shows the slight changes that occurred during a period of 21 days. Under other conditions Allyn and Baldwin (*2*) show significant time-potential changes in sterile media of various other types. The differences noted might perhaps be accounted for by an incomplete seal over the closed

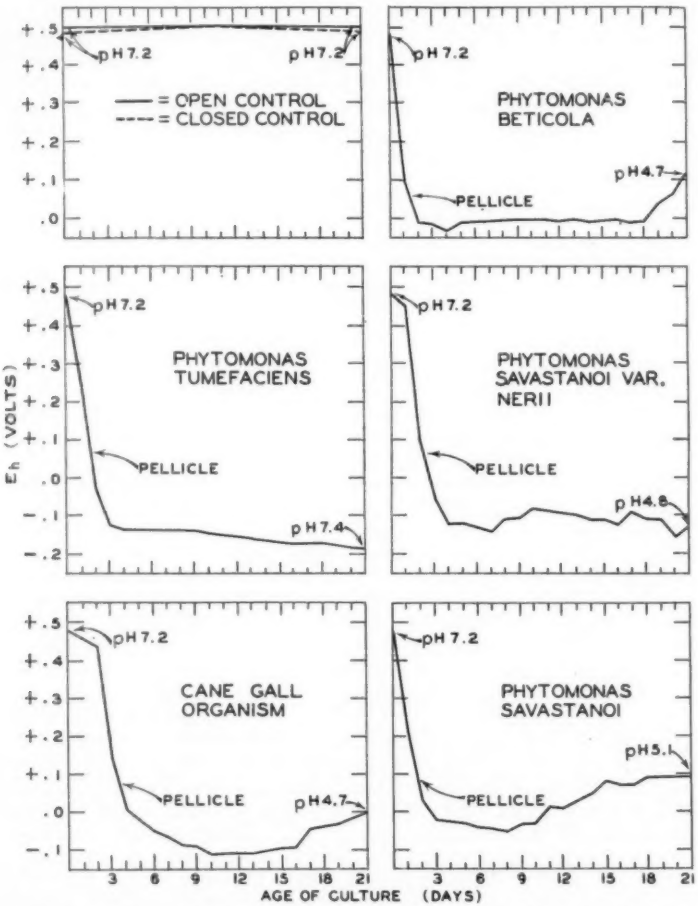

FIGURE 2.—Action of five gall-forming plant pathogens in relation to time on oxidation-reduction intensity, measured in volts (E_h), change in pH, and pellicle formation in yeast-extract-glucose-mineral-salt liquid medium at 24° C.

tubes (fig. 2), or by the use of yeast extract by the writer which had either stood a long time or had been heated enough to destroy its capacity for further reduction.

Changes in hydrogen-ion concentration of the sterile basic medium were reflected in the platinum electrode potentials. Figure 3 shows

the observed E_h of their sterile basic medium at stated pH values. The readings through the physiological range of pH fall approximately on a straight line. For each unit change of hydrogen-ion concentration between pH 2.0 and 11.0 the average observed change of the platinum-electrode potential was approximately 48 millivolts. Clark and Cohen (*5*) in a study of the theretical effects of pH on E_h found for each unit change of pH a possible shift of from 0 to more than 60 millivolts in the physiological range. Obviously oxidation-reduction systems are comparable only when hydrogen-ion concentrations are considered.

Autoclaving the platinum electrodes in distilled water was observed to increase their potentials in the basic medium over clean but unsterilized electrodes. A change of 50 to 60 millivolts toward the more oxidized region was noted. Similar changes were noted by Allyn and Baldwin (*2*) between electrodes autoclaved in steam and in water.

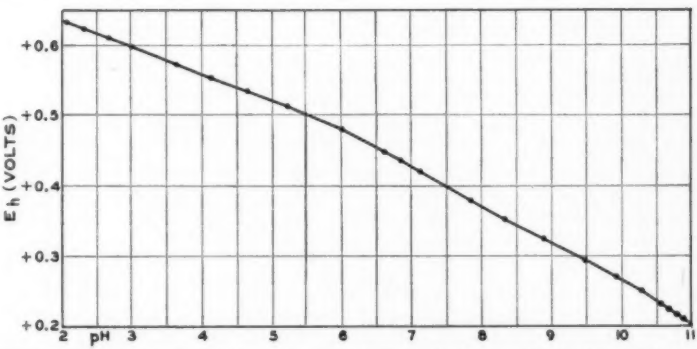

FIGURE 3.—The relation between hydrogen-ion concentration and oxidation-reduction intensity in yeast-extract-mineral-salts-glucose liquid medium at 24° C. This curve was obtained by titrating the medium with standard hydrochloric acid in the acid range and with sodium hydroxide in the alkaline range. The E_h and pH were read potentiometrically with the platinum and glass electrodes described in the text.

The use of more than one electrode was obviously necessary since an erratic behavior of electrode potentials was sometimes noted in cultures growing in the basic medium. Oftentimes only one electrode was affected. After a few hours or days the two electrodes would again be in perfect agreement and follow the usual course. The cause of these peculiar variations was not determined.

TEMPERATURE RELATIONS

Comparative thermal relations of these organisms were studied by measuring the diameter of giant colonies at stated temperatures on yeast-extract glucose agar after 14 days of incubation. The cultures were grown in 6-ounce screw-cap bottles which contained a thick layer of agar adjusted to pH 7.0. Needle pricks were used to start the colony on the surface of the substrate. The average diameter of the colonies in millimeters was taken as an index of growth at temperatures between 4° and 36° C.

The results of this study are presented graphically in figure 4. The region of optimum growth under the conditions of this experiment for all of these cultures, except the beet gall organism, occurred at 28° C. For all practical purposes this temperature can also be considered satisfactory for the beet gall organism, since only a slight increase of colony size over 28° was recorded for its observed optimum of 32° and since this difference is within experimental error. It is interesting, physiologically, that this group of bacteria should have similar thermal relations in culture.

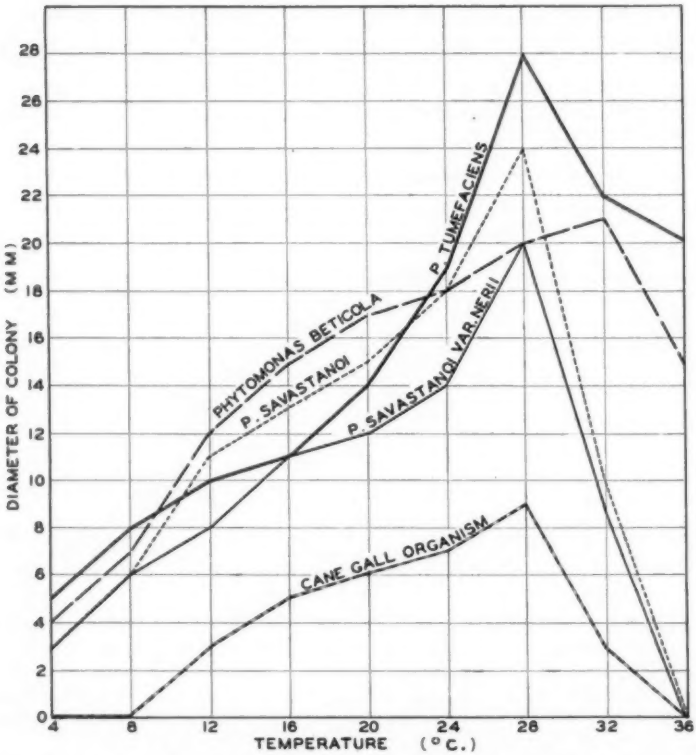

FIGURE 4.—Growth of various gall-forming plant pathogens as measured by colony diameter on yeast-extract-glucose-mineral-salts agar at stated temperatures during 14 days

HYDROGEN-ION RELATIONS

The maximum and the minimum hydrogen-ion concentration at which growth was initiated were studied in the basic medium containing glucose. Sodium hydroxide or hydorchloric acid was added to the media to shift the hydrogen-ion concentration to the limits of growth. The alkaline media were stored over strong alkali to prevent carbon dioxide of the air from changing the initial reaction. This procedure was only partly successful, however, because any

growth produced by the organisms tended to acidify the medium. Only the rough alkaline limits could be determined by this method because the alkaline media gradually shifted toward neutrality and after a few days or a week bacterial growth was invariably initiated in all tubes. No trials were made in a more highly buffered medium.

The results of the studies revealed that the crown gall, cane gall, and olive knot bacteria were similar in the acid regions and were prevented from establishing growth between the ranges of pH 4.2 and 4.4; while the oleander gall organism was unable to establish growth at pH 3.8 to 4.0; and the beet gall organism, at pH 3.6 to 3.8. The cultures were incubated 21 days at 24° C. The alkaline tolerance of the group could only be approximated, as previously explained. Indications up to 1 week pointed to the pH region of 9.5 to 10.5 for the whole group as the limit of alkaline tolerance under the conditions of this experiment. Figure 3 shows the E_h of the sterile medium at various hydrogen-ion concentrations. Doubtless the E_h was an important influence, as well as pH at the limits of growth.

UTILIZATION OF NITROGEN AND CARBON COMPOUNDS

The ability of the gall-forming bacteria to utilize selected sources of nitrogen and carbon in a mineral-salt medium was examined to learn more of the comparative physiology of these organisms and to determine, if possible, any similar or dissimilar metabolic characteristics. These characters were measured by (1) the relative amount of growth produced in the tubes, (2) the change in titratable acidity, and (3) the final hydrogen-ion concentration of the culture.

UTILIZATION OF VARIOUS NITROGENOUS COMPOUNDS

The various media for studying nitrogen utilization were made by substituting the nitrogen compounds listed in table 3 for the yeast extract of the basic medium. Concentrations of 0.5 percent by weight were used in these media except in the case of l-cystine, uric acid, and l-tyrosine which were employed in 0.1 percent concentrations. Traces of available food materials (probably nitrogenous) were detected in the distilled-water supply during preliminary studies. These impurities of the water were removed by redistillation over potassium permanganate.

The comparative growth of these organisms in various nitrogenous media containing respectively various nitrogenous compounds is summarized in table 3. The crown gall organism appeared to use many and diverse, simple and complex compounds while the cane gall organism used the more complex compounds. The other cultures were apparently intermediate. Of the 20 media tested in this experiment 9 were used by all of the organisms. Imide, amide, amino, and organic ammonium nitrogen characterize the compounds utilized by all of the organisms.

The comparative titratable acidity or alkalinity induced by these organisms in various nitrogenous media is also summarized in table 3. Since detailed treatment of the data would require too much space, only the more outstanding variations are noted. The crown gall bacteria produced marked quantities of total alkalinity in urea and l-asparagine media. The cane gall bacteria produced large

TABLE 3.—*Growth, hydrogen-ion concentration, and change in titratable acidity produced by five gall-forming bacteria in glucose-mineral-salt media containing various nitrogen sources*

Nitrogen source [1]	Growth [2] of organism causing—					Control tubes	pH concentration with organism causing—					Control tubes [3]	Change in titratable acidity [4] with organism causing—				
	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot		Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot		Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot
						pH						*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Potassium nitrate	+++	0	++	+++	+++	6.5	7.1	6.4	4.0	4.7	4.1	0.05	−0.05	+0.15	+0.15	+0.25	+0.15
Ammonium nitrate	++	0	+	0	0	6.6	4.7	5.9	3.9	5.3	5.5	.70	+.60	+.30	+.40	+.10	+.10
Ammonium sulphate	++	0	+	+	0	6.8	4.5	6.0	3.8	5.5	5.8	1.00	+.80	.00	.00	+.10	.00
Ferric ammonium citrate	+++	+	+++	+++	+++	7.1	7.8	6.0	5.9	7.6	7.7	1.06	−.14	+.14	+.94	−.36	−.16
Potassium nitrite	+	0	0	0	0	6.9	7.3	7.0	7.8	8.0	7.7	.40	−.20	−.35	−.10	−.10	−.10
Uric acid	+++	+	+++	+++	+++	6.7	6.6	3.9	4.7	6.0	6.5	.15	+.15	+.05	+.15	+.05	+.15
Urea	++	0	+	+++	+++	6.2	8.8	7.0	3.3	8.6	9.0	.60	−1.20	−.35	+.10	−.20	−.10
Dicyandiamide	+	0	0	+	+	7.0	5.1	7.1	7.2	4.2	4.1	.06	+.24	−.04	−.04	+.04	+.12
Oxamide	+++	+	++	+++	++	7.0	4.7	4.7	3.8	4.1	4.5	.34	+.46	+.46	+.76	+.36	+.06
Acetamide	++	0	+	+	++	7.0	4.8	6.7	3.0	5.3	4.0	.10	+.50	.00	+.35	+.30	+.15
Succinimide	+++	+	+++	+++	+++	7.0	7.4	4.7	3.2	5.8	5.1	.50	−.10	+2.00	+1.15	+.40	+.10
l-asparagine	+++	+	+++	+++	+++	7.1	8.5	4.6	5.3	8.8	8.6	1.20	−1.00	+.80	+1.50	−1.60	−1.20
Barbital sodium	0	0	0	+	+	6.8	6.8	6.6	6.6	4.2	4.5	2.30	+.70	+.70	+.30	+.30	+1.30
Guanidine carbonate	0	0	0	0	0	6.5	8.2	8.0	8.1	8.0	8.0	.40	−.40	−.25	−.40	−.40	−.25
Glycine	0	0	+	+++	+++	6.9	6.7	6.7	3.8	8.0	8.0	.50	+.10	+.10	+.80	−.50	−.10
l-tyrosine	+++	+	+	+++	++	6.8	3.8	3.4	3.0	3.8	3.5	.15	+.15	+.35	+.45	+.25	+.30
l-cystine	++	+	+	+++	++	7.0	4.7	5.0	3.1	4.0	3.8	.20	+.30	+.10	+.80	+.30	+.40
d-glutamic acid	+++	+++	+++	+++	+++	7.0	6.9	4.8	4.4	4.3	3.6	.15	+.25	+1.05	+.15	+.15	+.85
Yeast extract	+++	+++	+++	+++	+++	7.0	6.4	3.7	3.2	3.6	3.9	.13	+.17	+.57	+.07	+.47	+.57
None	0	0	0	0	0	7.0	6.7	6.8	6.9	6.9	6.2	.10	.00	+.05	.00	.00	+.10

[1] Neutralized with sodium hydroxide, as explained in the text.
[2] Amount of growth indicated as follows: 0, none or trace; +, slight; ++, moderate; +++, abundant. The cultures were incubated 20 days at 24° C.
[3] Cubic centimeters of 1/10 N sodium hydroxide necessary to bring 10 cc of the sterile medium to the neutral point of phenolphthalein.
[4] In each case the increase (+) or decrease (−) in total acidity compared to the control tubes is recorded in cubic centimeters of 1/10 N sodium hydroxide or hydrochloric acid. Phenolphthalein was used as the indicator.

quantities of acid in succinimide, and d-glutamic acid media. The beet gall bacteria produced considerable acid especially in succinimide and l-asparagine media and notable amounts in several others. The oleander gall and olive knot organisms were similar in most of the compounds tested and resembled the crown gall bacteria in the production of an alkaline reaction from l-asparagine. The only consistent relation among these cultures, when they grew, appeared in the total acidity induced in media made with uric acid, oxamide, l-tyrosine, l-cystine, and yeast extract.

The comparative hydrogen-ion concentrations induced in these various nitrogenous media are summarized in table 3. Some of these media, as already mentioned, supported visible growth of all five cultures. Among such media those made with oxamide, l-tyrosine, and l-cystine became acid with each organism. All the organisms which grew and induced similar reactions, produced acid in media made with acetamide, barbital sodium, ammonium nitrate, and ammonium sulphate. No comparable alkaline reactions were induced. These results should be considered in relation to the total acidity discussed earlier.

Oxidation-reduction intensities of these media were measured before seeding and again after an incubation period of 21 days at 24° C. Detailed treatment of these data is beyond the scope of this paper. As much as 0.05-volt drift was noted in some of the sterile media during the incubation period. The maximum and minimum E_h limits of all of the sterile media lay within E_h+0.50 volt and +0.39 volt which represents rather oxidized conditions but which were within the limits of growth in the basic medium. The inability of certain organisms to grow in some of the media cannot be explained by an unfavorable initial oxidation-reduction potential. Yeast-extract-glucose liquid medium, one of the most favorable for growth, started at E_h+0.44 volt. Others ranged from +0.39 to +0.50 volt. The final electrode potentials of the cultures were commonly within this same range. However, there were some notable exceptions many of which appeared when the culture had a relatively strong alkaline reaction. The consideration of these E_h readings must take into account both the facts (1) that the E_h readings represent the resultant of the oxidation-reduction conditions and the hydrogen-ion concentration, and (2) that the oxidation-reduction conditions become rapidly more oxidizing at the end of the active growth period.

UTILIZATION OF VARIOUS SOURCES OF CARBON

Preparation of the various media employed in the utilization of carbon was made by substituting the carbon compounds listed in table 4 for the glucose of the mineral-salt-yeast-extract basic medium.

The comparative growth of the gall-forming bacteria in media containing various sources of carbon is given in table 4. A trace of growth appeared in the yeast extract which prevented a clear-cut picture of utilization as compared with no utilization of the compound in question. The use of yeast extract as a source of nitrogen was essential for growth of all the organisms studied. It was clear that most of the carbon media tested were available to the needs of the bacteria. The notable exception appeared to be oxalic acid.

TABLE 4.—*Growth, hydrogen-ion concentration, and change in titratable acidity produced by five gall-forming bacteria in yeast-extract-mineral-salt liquid media containing various carbon sources*

Carbon source [1]	Growth [2] of organism causing—					Control tubes	pH concentration with organisms causing—					Control tubes [3]	Change in titratable acidity [4] of organism causing—				
	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot		Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot		Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot
						pH						*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
l-arabinose	++	++	++++	++++	++++	6.7	6.3	4.1	4.2	7.3	6.1	0.50	+0.25	+0.70	+0.50	0.00	+0.30
l-xylose	++	++	++++	++++	++++	6.7	6.3	4.1	4.6	7.0	4.3	.50	+.25	+.90	+.60	+.10	+.80
l-rhamnose	+++	+++	++++	+	+	7.2	6.5	5.6	4.7	7.4	7.0	.08	+.12	+.32	+.32	−.02	+.12
d-fructose	+++	++	++++	++++	++++	6.4	6.2	5.1	4.6	4.7	4.2	.30	.00	+.20	+.40	+.20	+.40
d-mannose	+++	++	++++	++++	++++	6.7	6.5	4.9	4.6	6.4	5.8	.50	+.10	+.40	+.45	+.60	+.10	+.70
d-galactose	++	++	++++	++++	++++	6.6	6.2	4.0	3.2	3.8	3.7	.40	.00	+.40	+.60	+.60	+.30	+.70
d-glucose	+++	+++	++++	++++	++++	6.6	6.3	3.8	3.4	4.0	4.0	.30	.00	+.40	+.80	+.80	+.20	+.40
Sucrose	++	++	++++	++++	++++	7.0	7.5	7.0	4.5	7.8	8.3	.20	−.10	+.10	+.20	−.10	−.20
Maltose	++	++	++++	++++	++++	7.2	7.6	7.2	5.2	8.4	8.2	.20	−.10	.00	+.20	−.20	−.10
Lactose	++	++	++	++++	++++	6.8	6.6	4.4	6.4	8.0	8.0	.40	−.10	+.30	+.10	−.30	−.30
Melezitose	+++	++	++	+	+	6.7	7.0	7.8	7.9	8.0	8.0	.10	−.04	−.02	.00	−.05	−.05
Raffinose	++++	++++	++++	++	++	6.8	6.8	6.8	8.0	8.1	8.0	.10	+.05	+.05	.00	−.02	−.05
Starch	++++	++++	++++	++++	++++	6.5	8.2	8.1	8.1	8.0	8.0	.40	−.30	−.40	−.30	−.30	−.30
Inulin	++++	++	++	++	++	6.7	6.7	8.0	8.2	8.1	8.1	.30	.00	−.20	−.20	−.20	−.20
Dextrin	++++	++	++++	++	++	7.3	7.0	7.2	6.1	8.1	8.0	.10	+.05	.00	+.20	.00	.00
Pectin	++++	++	++++	++++	++++	6.3	7.5	7.2	7.7	6.4	7.8	.40	−.20	−.20	−.30	−.30	−.30
Glycerol	++++	++++	++++	++++	++++	7.1	6.6	7.1	5.6	6.3	6.8	.05	+.15	+.05	+.25	+.05	+.15
Erythritol	+	+++	+	++	+	7.0	7.9	4.2	8.0	8.1	8.1	.06	−.03	+.33	.00	.00	−.06
Lactositol	++++	++++	++	++	++	7.2	6.9	7.3	8.1	8.1	8.2	.05	+.15	−.15	−.05	−.05	−.05
Mannitol	++++	++++	++++	++++	++++	7.3	6.4	7.3	4.2	6.6	6.5	.05	+.15	+.15	+.45	+.15	+.15
Dulcitol	++++	++	++	++	+	7.1	6.8	7.1	8.0	8.1	8.1	.10	+.06	+.05	.00	−.05	−.05
Salicin	++++	++++	++++	+	+	7.4	6.2	7.1	4.4	8.2	8.2	.10	+.20	+.10	+.40	.00	.00
Aesculin	++++	++	++	+	++++	6.6	6.6	6.7	5.3	7.0	6.9	1.90	+.01	+.20	+.10	−.10	−.10
Phloridzin	++++	++	++++	++	++	6.8	6.8	7.1	7.0	6.9	7.1	1.50	+.30	.00	−.20	−.10	−.20
Calcium gluconate	++++	++	++++	++++	++++	6.3	7.5	7.4	5.8	7.7	7.5	.25	−.15	−.15	+.75	−.15	−.15
Formic acid	++++	+	+	+	++	8.0	9.2	8.8	9.1	9.3	9.4	.05	−.45	−.15	−.35	−.50	−1.35
Acetic acid	++++	++	++++	++++	++++	7.0	9.0	8.1	8.4	9.1	8.8	.10	−.40	.00	−.05	−.35	−.10
Propionic acid	++++	++	++++	++++	++++	7.3	8.9	8.0	8.4	8.1	8.8	.10	−.10	−.02	.00	−.50	−.40
Glycollic acid	++	++	+++	+	+	7.3	8.1	8.3	7.9	8.0	8.0	1.00	−.15	−.05	−.05	.00	−.07
Malonic acid	++	++	++++	++	++++	6.8	8.0	8.0	9.1	8.7	9.3	.30	−.20	−.20	−.70	−.30	−1.10
Oxalic acid	+	0	+	+	+	7.4	8.0	7.4	8.2	8.3	8.2	.08	−.02	+.02	−.02	−.03	−.06
Succinic acid	++	++	++++	++++	++++	7.3	9.4	8.6	9.3	9.2	9.2	.15	−.75	−.10	−.75	−.57	−.45
Tartaric acid	+	+	+	++++	++++	7.3	8.0	8.1	8.2	9.2	9.1	.08	−.01	−.04	−.02	−.58	−.37
Malic acid	+++	++	++++	++++	++++	7.0	9.2	8.7	9.2	9.5	9.4	.15	−.85	−.15	−.85	−2.05	−1.25
Citric acid	++	+	++++	++++	++++	7.2	8.2	7.2	9.2	9.4	9.0	.10	−.05	−.20	−.70	−.50	−.30
None (yeast extract only)	+	+	+	+	+	7.0	7.9	8.0	7.9	8.1	8.1	.00	.00	.00	.00	.00	.00

[1] Neutralized with sodium hydroxide as explained in the text.
[2] Amount of growth indicated as follows: 0, none; +, slight; ++, moderate; +++, abundant. The cultures were incubated 21 days at 24° C.
[3] Cubic centimeters of 1/20 N sodium hydroxide necessary to bring the sterile medium to the neutral point of phenolphthalein.
[4] In each case the increase (+) or decrease (−) in total acidity compared to the control tubes is recorded in cubic centimeters of 1/20 N sodium hydroxide or hydrochloric acid. Phenolphthalein was used as the indicator.

Of the 35 compounds tested, 24 were undoubtedly used by all of the organisms to some extent, as indicated by the turbidity produced over and above that of the control tubes.

The comparative titratable acidity or alkalinity developed in these media are also summarized in table 4. Consistent production of approximately the same amount of acid or alkaline materials by all these organisms residing in the same medium was not observed except in limited instances. Each of the organisms yielded values approximately similar to those induced by the others in media containing the following sources of carbon: Melezitose, raffinose, starch, inulin, pectin, glycerol, and glycollic acid. The total acidity values of these media differed slightly among themselves.

The comparative hydrogen-ion concentrations induced by these organisms in the various carbon-containing media are shown in table 4. Where there was little or no growth, there was often no important change. At least slight acid reactions were induced by all of the organisms in d-fructose, d-glucose, d-mannose, and d-galactose. Varying acid to neutral reactions were induced by all of the organisms in l-xylose, aesculin, glycerol, phloridzin, and mannitol. Alkaline reactions were induced by all of the organisms in melezitose, starch, yeast extract, and in all of the sodium salts of the organic acids studied. The alkalinity developing from the latter compounds is probably explained by release of the alkaline cation following utilization of the organic radical of the molecule. A slight alkalinity was induced in the yeast extract medium which contained no added source of carbon. In media such as that containing oxalic acid, only a slight growth was induced accompanied by an alkaline reaction, which apparently came from the yeast extract. For the most part the crown gall organism produced little, if any, acid from the various sources of carbon. The cane gall and beet gall organisms produced acid from several of the sugars while the oleander and olive knot organisms had a tendency to produce an alkaline reaction. The classification used is highly artificial and is presented merely for convenience.

The average E_h limits of the sterile control tubes fell between E_h +0.48 and E_h+0.43 volt except in sodium formate which was +0.17 volt. No appreciable drift was noted during the incubation period (21 days at 24° C.) in any of the sterile media. The media containing various carbon sources was found to be poised at a satisfactory potential for growth of these bacteria at the beginning of the experiments. In the case of the crown gall bacteria slight reducing conditions were found in all media except that containing phloridzin. With regard to the remaining pathogens, low E_h values were found in most of the carbon-containing media except where strong acid reactions developed. A consideration of these E_h readings must take into account the pH relationships mentioned earlier, the time-potential curves mentioned later, and the changes incurred by cessation of growth. Detailed treatment of these results are beyond the scope of this paper.

Parts of earlier work by Brown (*4*), Riker (*14*), C. O. Smith (*18*), and E. F. Smith (*19*) have been repeated and confirmed by the present studies in which the various organisms have been examined in parallel cultures.

Quantitative fermentation studies of reducing sugars were made according to the method of Stiles et al. (*22*). One-half percent sugar solutions, in 10-cc lots were used as noted previously.

TABLE 5.—*Action of certain cell-stimulating plant pathogens on reducing sugars as measured by quantitative determinations of reducing substances remaining in the cultures after fermentation for 21 days at 24° C.*

Carbon source	Sugar fermented [1] by organisms causing—				
	Crown gall	Cane gall	Beet gall	Oleander gall	Olive knot
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
d-glucose	31	29	55	49	37
d-galactose	35	22	65	41	35
d-mannose	42	25	80	20	34
d-fructose	67	40	89	30	59
l-xylose	35	26	73	9	50
l-arabinose	62	29	68	11	46
Sucrose	41	23	79	31	2
Maltose	47	50	85	73	64
Lactose	16	[2] 0	24	[2] 0	[2] 0

[1] Control tubes analyzed as 100 percent.
[2] Slight fermentation was probable but not sufficiently abundant to measure successfully.

The results of analysis of total reducing substances in the culture after the usual incubation period (21 days at 24° C.) are summarized in table 5. Maltose and d-fructose were fermented in the largest quantities by most of the organisms studied. No utilization of lactose could be found in the case of cane gall, oleander tubercle, or the olive knot bacteria. The oleander organism fermented l-xylose and l-arabinose only slightly; sucrose was scarcely used by the olive knot organism. Moderate turbidity was noted in all these sugar solutions. It should be mentioned that large-bore test tubes gave a higher percentage of fermentation than did small tubes, although the same quantities of media were used. All the tubes were capped with tin foil to prevent evaporation; consequently, the degree of fermentation was probably less than it would have been in fully aerated cultures. However, the fact that certain sugars were used appears clearly in these results. Their utilization might be questioned in some cases when only acid production was considered.

OXIDATION-REDUCTION STUDIES IN LIQUID CULTURES

Comparative time-potential measurements were made in yeast-extract-glucose-mineral-salts liquid media, the technic used being similar to that of Allyn and Baldwin (*2*). The platinum electrodes were sterilized in distilled water and gave a potential of approximately +0.48 volt at pH 7.2 in the sterile basic medium at the beginning of the experiment. Daily readings were made on undisturbed cultures. The results are plotted in figure 2. The final values of the electrode potentials represent an average reading of four electrodes since each experiment was made in duplicate. Although the work was repeated three times, only the results of one representative experiment are shown. The results in the other two trials were similar but are omitted because of their volume.

Characteristic drop in the E_h value was observed in all the cultures during the first 3 days. The velocity of change was apparently governed by the rate of growth since the cane gall bacteria were

consistently slow as compared with the other organisms. No correction was made for the pH effect. The influence of pH upon the E_h reading has already been examined (fig. 3) in the basic medium employed. It is interesting to observe that, when the bacteria formed acid which alone would raise the E_h values, nevertheless, the metabolism of the organisms lowered the E_h readings. Even though these cultures apparently behaved alike in this experiment, it should be noted that other organisms (*2*, *7*, *17*) show similar curves. However, it appears altogether likely that certain organisms, when capable of growing in plant tissue, may exert a distinctly reducing effect. Further studies in this field were beyond the scope of this paper.

DISCUSSION

Some differences in the comparative physiology of the above-mentioned organisms (tables 1 and 2) appeared during identification, although the oleander and olive knot cultures were closely related. Among these five cultures, that from beet gall exhibited the widest differences in that it was pigmented, caused slight digestion of casein, slight hydrolysis of starch, and slight liquefaction of gelatin. In temperature studies its optimum of 32° C. was several degrees above that of the other cultures. All the cultures, except those of the oleander and olive knot, were different from each other in litmus milk. All of the cultures were parasitic on the hosts from which they were isolated. Only the crown gall organism infected all the hosts employed.

A suggestion of nitrogen fixation similar to that reported earlier (reviewed by Riker and Berge (*15*) was found in preliminary experiments, but later a small amount of utilizable material was detected in the distilled water. In further experiments the water was redistilled over potassium permanganate which removed the utilizable material. In the nitrogen-free (except atmospheric nitrogen) media no growth occurred, which shows the inability of these organisms to fix much, if any, atmospheric nitrogen in these cultural media. Consequently, reports that nitrogen fixation is common in laboratory media may be open to question, and suggestions concerning the cause of cell stimulation based on nitrogen fixation seem to have an inadequate experimental background.

In the studies on the utilization by these organisms of materials containing carbon and nitrogen, valid measurements on which they could be compared appeared to be growth, hydrogen-ion concentration induced, and change in titratable acidity. Titratable acidity was commonly, but not always, reflected in the reaction. Change of oxidation-reduction potentials appeared of doubtful value as a measure of carbon or nitrogen utilization. Since all the organisms studied induce plant overgrowths, and although the possibility is recognized that they may induce the stimulation in different ways, it would seem that similarities in their physiology are of more importance than dissimilarities when considered in relation to the general question of atypical and pathological multiplication of plant cells. Consequently emphasis is placed on characters which the organisms have in common. The following list shows the similarities in growth and reaction induced by these organisms in media with various sources of carbon and nitrogen. The crown gall and beet gall organisms showed the least similarities encountered, whereas the oleander and

olive knot organisms showed the greatest similarities in that they grew and produced comparable reactions in 46 of the 54 compounds tested.

All organisms studied

Starch
Pectin
Phloridzin
Formic acid
Acetic acid
Propionic acid
Glycollic acid
Malonic acid
Succinic acid
Tartaric acid
Malic acid
Oxamide
l-tyrosine
l-cystine

Crown gall and cane gall organisms [4]

Sucrose
Maltose
Raffinose
Dextrin
Glycerol
Lactositol
Dulcitol
Aesculin
Calcium gluconate

Crown gall and beet gall organism [4]

Lactose
Erythritol
Oxalic acid
Citric acid

Crown gall and oleander gall organism [4]

d-mannose
Sucrose
Glycerol
Erythritol
Mannitol
Calcium gluconate
Oxalic acid
Citric acid
Ferric ammonium citrate
Uric acid
l-asparagine

Crown gall and olive knot organisms [4]

l-arabinose
l-rhamnose
Glycerol
Erythritol
Mannitol
Aesculin
Calcium gluconate
Oxalic acid
Citric acid
Ferric ammonium citrate
Uric acid
l-asparagine

Cane gall and beet gall organisms [4]

l-arabinose
l-xylose
l-rhamnose
d-fructose
d-mannose
d-galactose
d-glucose
Melezitose
Inulin
Ferric ammonium citrate
Uric acid
Succinimide
l-asparagine
d-glutamic acid
Yeast extract

Cane gall and oleander gall organisms [4]

d-fructose
d-galactose
d-glucose
Inulin
Aesculin
Calcium gluconate
Succinimide
d-glutamic acid
Yeast extract

Cane gall and olive knot organisms [4]

l-xylose
d-fructose
d-galactose
d-glucose
Inulin
Glycerol
Aesculin
Calcium gluconate
Succinimide
d-glutamic acid
Yeast extract

Beet gall and oleander gall organisms [4]

d-fructose
d-galactose
d-glucose
Raffinose
Inulin
Erythritol
Lactositol
Dulcitol
Citric acid
Potassium nitrate
Acetamide
Succinimide
d-glutamic acid
Yeast extract

Beet gall and olive knot organisms [4]

l-xylose
d-fructose
d-galactose
d-glucose
Raffinose
Inulin
Lactositol
Erythritol
Dulcitol
Citric acid
Potassium nitrate
Acetamide
Succinimide
d-glutamic acid
Yeast extract

Oleander and olive knot organisms [4]

Rhamnose
d-fructose
d-galactose
d-glucose
Sucrose
Maltose
Lactose
Melezitose
Raffinose
Inulin
Dextrin
Glycerol
Erythritol
Lactositol
Mannitol
Dulcitol
Salicin
Aesculin
Calcium gluconate
Citric acid
Potassium nitrate
Ferric ammonium citrate
Uric acid
Urea
Dicyandiamide
Acetamide
Succinimide
l-asparagine
Barbital-sodium
Glycine
d-glutamic acid
Yeast extract

[4] In addition to compounds used by all organisms studied.

Certain reactions induced are particularly noteworthy. Utilization of the sodium salts of the organic acids together with the production of alkali was found with all the cultures. Similarly, oxamide, l-tyrosine and l-cystine were used by all the cultures, but with the formation of acid. With many other substances varying degrees of differences in the reaction induced indicate that neither simple acidity nor simple alkalinity should be considered the direct factors of host-cell stimulation.

Although the production of an acid or alkaline reaction in sugar media is a common indication of its utilization, it is unreliable if neutral products are formed. Therefore, quantitative determinations of reducing substances remaining in the cultures after fermentation were made and are given in table 5. Lactose was utilized sparsely by all of the cultures, and sucrose was not fermented to any extent by the olive knot organism. l-xylose was not appreciably utilized by the oleander gall organism. It would seem that lactose and l-xylose could be included with many other substances to be set aside during studies of materials that may contribute to the development of stimulating factors until certain others have received more attention.

The hydrogen-ion activities at levels in which growth does not occur in the basic medium appears much more significant when the interrelationship of pH and E_h are considered together (fig. 3). Shifting the pH from 4.0 to 10.0 with sodium hydroxide involved a change in E_h of nearly 312 millivolts in the basic medium. This range of voltage is narrow as compared with observed ranges caused by cell proliferation in undisturbed media (fig. 2). Under these conditions the E_h range of growth was approximately 600 millivolts. If it is true that the alkaline limit of growth lies in the region of pH 10.0 than the E_h of the basic medium (+0.265 volt) approaches an optimum at this level (fig. 2), and the pH then becomes an inhibiting factor of growth. In the acid region near pH 4.0 both the hydrogen-ion activity and the E_h (+0.556 volt) approached the limit of growth, and either one or the combination may, therefore, be the limiting factor. In limited unpublished experiments, an unsuccessful attempt was made to separate these two apparently limiting factors by poising the basic medium at stated E_h-pH levels by using common oxidizing or reducing agents in the medium. Certain indications appeared suggesting that the limiting oxidation-reduction range of this group of cultures lay near +0.6 volt on the oxidized side of the normal hydrogen electrode and near −0.3 volt on the reduced side, which suggests that pH was the limiting factor in the acid region. Furthermore, the concentration of the reagents used to reach these physical limits had undesirable effects upon the medium. These studies indicate the great practical importance of considering E_h as well as pH for the growth of these pathogenic microorganisms in culture. No doubt it is very important also for the growth of microorganisms in the plant and has a bearing on both susceptibility and resistance.

That the oxidation-reduction potential of a medium may play a significant role in the ability of aerobic organisms to grow in certain media has been shown by Allyn and Baldwin (*1*, *2*). The inability of an organism to utilize certain nutrients may, therefore, be a function of E_h. In order to determine whether this factor, as well as pH, was operating to prevent growth in the media used, tests were made

on the oxidation-reduction potentials developed in the media containing various sources of carbon and nitrogen before seeding and after incubation. These results, while not reported in detail, indicated that none of these media were beyond the range of E_h suitable for the growth of these organisms. Final potential readings were of greater oxidation intensity than the final readings of the time-potential measurements of undisturbed liquid cultures in the basic glucose medium. These results are attributed largely to cessation of growth and to auto-oxidation before the final readings were taken. In accord with figure 3, the production of an acid reaction in the medium seemed to be correlated with relatively strong oxidizing potentials, and, conversely, relatively strong reducing potentials were apparently correlated with alkaline reactions. Sodium formate gave the most reducing readings of all of the compounds in this study both before and after incubation. In this case the difference in E_h was clearly more than could be accounted for on the basis of pH differences.

Studies on electrode potentials developed in liquid cultures in relation to time (fig. 2) showed, for all of the organisms, increased reducing potentials with increase of time, regardless of hydrogen-ion concentration. A study of the nature of the factors causing this reduction was not undertaken. Since other unrelated organisms show similar results, this characteristic is not peculiar to the gall-forming group. If these cell-stimulating bacteria bring about a relatively reduced potential within the host tissue as was found in culture, this change would probably influence the unknown factors that stimulate growth. However, Hendrickson et al. (*7*) and Sagen et al. (*17*) found that the reducing action of nonpathogenic cultures was apparently similar to that of pathogenic cultures.

It appears in the data presented not only that physiological similarities and dissimilarities among certain cell-stimulating bacteria have been clarified, but also that there are certain implications concerning factors (reviewed *(15)*) possibly important in relation to atypical and pathological multiplication of cells.

SUMMARY

Cross inoculations, with small pieces of gall tissue and with single-cell cultures of *Phytomonas tumefaciens*, *P. beticola*, *P. savastanoi* var. *nerii*, *P. savastanoi*, and an unnamed organism causing raspberry cane gall, made on tomato, garden beet, oleander, olive, and raspberry showed (1) that *P. tumefaciens* was pathogenic on all of the hosts used, (2) that *P. savastanoi* var. *nerii* was pathogenic on the oleander and olive, and (3) that each of the other organisms was pathogenic only on the host from which it was isolated.

Routine physiological studies on certain common bacteriological media showed that each of these bacterial organisms gave characteristic growth reactions which were distinctive for each organism, except the oleander and olive knot bacteria which showed similar cultural characters in all these media.

The optimum temperature range for colony diameter on agar was about 28° C. for all of these micro-organisms except *Phytomonas beticola* which produced its largest colonies at 32°.

The hydrogen-ion concentration at which growth was prevented in liquid culture was found to vary from pH 3.6 to 4.4 in the acid range and approximately 9.5 to 10.5 in the alkaline range.

Comparative utilization of several types of compounds indicated that oxamide, l-tyrosine and l-cystine were the only sources of nitrogen used in which all of the organisms distinctly produced similar reactions. Hydrogen-ion determinations were all in the acid range.

Comparative utilization of several types of compounds containing carbon indicated that starch, pectin, phloridzin, and the sodium salts of formic, acetic, propionic, glycollic, malonic, succinic, tartaric, and malic acids were the only sources of carbon used in which all of the organisms distinctly produced similar reactions. Hydrogen-ion determinations were all in the alkaline range.

Supplementing these studies, quantitative determinations were made of reducing substances remaining after fermentation in the cultures containing the reducing sugars. Some of the cultures showed similar, and other unequal, utilization. In some cases little or no loss of sugar was detected by the analyses, although sugar fermentation was indicated by turbidity and by change of reaction.

The inability of certain of these organisms to utilize several of the compounds containing carbon or nitrogen was not caused by unfavorable oxidation-reduction intensities of these media at hydrogen-ion concentrations approaching neutrality.

Hydrogen-ion concentration of the sterile yeast-extract-glucose-mineral-salts liquid medium was found to have a marked influence on the oxidation-reduction intensity of this medium. Strong acid reactions were accompanied by relatively strong oxidizing potentials, either or both of which may have limiting or at least unfavorable influences on growth in the extreme acid region.

Oxidation-reduction-potential measurements made at frequent time intervals with various growing cultures in a yeast-extract medium showed that all these organisms produced relatively strong reducing potentials in undisturbed liquid cultures. This occurred in opposition to the oxidizing action of acid metabolic products.

The similarities of these organisms are discussed in relation to their comparative physiology and its bearing on certain working hypotheses for the cause of atypical and pathological multiplication of plant cells.

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OVARIAN DEVELOPMENT IN CALVES [1]

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INTRODUCTION

The presence of follicles of approximately mature size in calves 5 to 12 weeks of age, reported by Heitz and confirmed by Kappeli and Küpfer, is referred to by Hammond.[2] The last-named writer states that the age of puberty is probably reached at about 9 months and comments upon the length of the interval from the appearance of large follicles to puberty.

It has been shown for the rabbit and the pig respectively (Hertz and Hisaw,[3] and Casida [4]) that the immature female does not react macroscopically to gonad-stimulating extracts of the pituitary until an age is reached when vesicular follicles are present in the ovary. The observations mentioned above indicate that very young calves may be suitable for experimentation on the effects of various gonad-stimulating extracts on the bovine ovary; furthermore, such animals are desirable for experimental work for economic reasons. A study of the degree of follicular development in heifer calves which had been slaughtered for veal was accordingly made to determine the suitability of such animals for experimentation and also to furnish data on normal variation in ovarian development.

MATERIAL AND METHODS

Genitalia from 190 heifer calves showing Holstein-Friesian color markings and from 83 calves of various other breeds (many of Guernsey coloring) were collected [5] and the weight of the carcass (dressed weight) obtained for each animal. These collections were made during February and March 1934; as far as could be determined the calves were from southern Wisconsin and northeastern Iowa.

The collected genitalia were brought to the laboratory, where determinations were made of the following characters of the right and left ovaries separately: Weight to the nearest decigram, number and diameter of visible vesicular follicles, number of blood follicles, and length, width, and depth of the ovary to the nearest millimeter. The external diameter of one uterine horn was also obtained at a level just above the external bifurcation.

Analytical balances were used in weighing the ovaries, and the follicle counts and measurements were made with a device similar to an egg candler, which passed a strong beam of light through the

[1] Received for publication Feb. 2, 1935; issued July 1935. Paper from the Departments of Genetics (no. 180) and Animal Husbandry, Wisconsin Agricultural Experiment Station.

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[5] The Oscar Mayer Packing Co., Madison, Wis., made possible the collection of this material.

ovary. The measurements were made with a vernier caliper. External diameters of the follicles were readily obtained if the follicles were protruding from the ovarian surface, but if the follicles were embedded it was necessary to measure the diameter of the translucent surface area produced by the follicular fluid. The dimensions of the ovary are those of the body only and do not include follicles which protruded markedly. The classification of blood follicles comprises a series ranging from large follicles which showed excessive vascularity and congestion in the follicular wall, with slight hemorrhage into the follicular liquor, to those approximately 1 mm in diameter, which appeared macroscopically to contain a bluish-red coagulum.

In the study of the data, calculations were made of the mean (M), standard deviation (σ), standard deviation of the mean (σ_M) or standard error, and simple correlation (r). Tests of the significance of the difference between means were based on Fisher's tables of the probability (P) distribution of t and x for different numbers of observations (N).[6] The significance of simple correlation coefficients, likewise, was tested by Fisher's table of values of the correlation coefficient for different levels of significance. The probability of equaling or exceeding by chance the different values of the constants was the criterion used for deciding upon their statistical significance. The significance of the difference between correlation coefficients was obtained by converting the values of r into z (Fisher's table of r for values of z) and then comparing the difference between the values of the two z's with the standard error of their difference. If the calculated value had a probability of chance occurrence of 0.05 or less it was considered significant, meaning that a figure of that value or greater would, on an average, occur 5 percent of the time by chance. When the figure was so large that the probability of occurring by chance was reduced to 1 percent, it was then considered to be statistically highly significant. These two points (0.05 and 0.01 of the normal probability curve) are approximately equal to 2 and 2½ times the standard deviation, respectively.

PRESENTATION OF DATA

A preliminary study showed that practically all of the ovaries contained vesicular follicles. Of the 190 Holstein calves, 98 percent had follicles 1 mm or more in diameter; 89 percent, follicles 4 mm or more in diameter; 41 percent, follicles 10 mm or more in diameter; and 3 percent, follicles as large as 13 mm in diameter. These calves ranged in dressed weight from 52 to 138 pounds. Considering the dressing percentage 65 (as estimated from records of the packing house), the live weight of these heifers was 80 to 212 pounds. This weight range, according to growth data (Brody et al.),[7] would indicate that the calves ranged from new-born to 14 weeks of age.

The mean dressed weight of the calves of other breeds was distinctly less than that of the Holstein calves (table 1). When the

[6] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London. 1930.

[7] BRODY, S., and others. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO DOMESTIC ANIMALS. Mo. Agr. Expt. Sta. Research Bull. 96, 182 pp., illus. 1926.

right and left ovaries were considered together as a unit (total ovarian complement) in these two groups, the mean values for all of the ovarian characters, except one (frequency of follicles 1–3 mm in diamter), were numerically greater in the other breeds than in the Holsteins. None of these mean differences, however, was statistically significant. The three characters for which the value of P was the lowest (chance difference least likely) were ovarian weight, frequency of follicles 4–13 mm in diameter, and volume of follicles 4–13 mm in diameter. Although the calves of other breeds were distinctly lighter in weight than the Holsteins and showed even greater ovarian development, the nonestimable effect of the breed variability in the former group makes any direct comparison with Holsteins on such a common basis as weight or estimated age seem unjustifiable.

TABLE 1.—*Comparison of calves of Holstein appearance with calves of various other breeds*

Item	Holstein $M \pm \sigma_M$	Other breeds $M \pm \sigma_M$	Probability of mean difference
Calves—number	190	83	
Dressed weight—pounds	88.6±1.1	82.8 ±1.8	<0.01
Uterine diameter—millimeters	10.43± .14	10.59± .17	.47
Ovarian weight [1]—grams	1.90± .09	2.15± .15	.14
Frequency of follicles of different diameters: [1]			
1 to 3 mm	36.8±3.6	35.1 ±6.0	.88
4 to 6 mm	3.34± .30	3.99± .44	.22
7 to 9 mm	1.05± .10	1.29± .14	.16
10 to 13 mm	.46± .04	.55± .07	.26
4 to 13 mm	4.86± .33	5.83± .45	.09
Total volume of follicles of different diameters: [1 2]			
4 to 6 mm—cubic millimeters	193± 17	234± 22	.14
7 to 9 mm—do	267± 25	316± 34	.24
10 to 13 mm—do	307± 32	339± 44	.50
4 to 13 mm—do	764± 42	888± 55	.08
Frequency of blood follicles	1.04± .12	1.34± .32	.36

[1] Combined right and left ovaries are considered as a unit for all ovarian characters in this table.
[2] No attempt was made to calculate total volume of follicles 1–3 mm in diameter because of inaccuracy in measurement of the smallest follicles.

Inasmuch as the Holstein calves represented a considerable range in age, as judged by the weight criterion, the association between dressed weight and the various ovarian characters should give some indication as to the type of morphological change which takes place in the ovaries during the first few weeks of postnatal life. Figure 1 shows this in terms of simple correlations. Of the 5 ovarian characters studied, only 3 (weight, frequency of follicles 4–13 mm in diamter, and volume of follicles 4–13 mm in diameter) were significantly correlated with dressed weight. The correlation between dressed weight and volume of follicles 4–13 mm in diameter was significantly higher than that between dressed weight and either of the other characters. Progressive ovarian development is the expression of physiological stimuli, even before puberty; therefore the best indices of the state of ovarian environment are weight of the ovaries and number and total volume of larger follicles, and the best index of all is the total volume of follicles 4–13 mm in diameter.

The mean total volume of follicles 4–13 mm in diameter is determined by the diameter and number of follicles in all calves and by the proportion of calves which have follicles of this size. The Holstein calves were divided into two weight groups (52–86 pounds and 87–138 pounds) of approximately equal frequency (table 2). Many

calves in each group had follicles at least as large as 4 mm in diameter, but the proportion of calves which had follicles of that minimum size was greater by 17 percent in the group of heavier animals. There was also a greater percentage of the heavier calves which had follicles approaching 13 mm in diameter; in addition there was a greater proportion of these heavier calves with more than one large follicle. The percentage of calves having more than 50 follicles less than 4 mm in diameter and also the percentage having blood follicles are very similar in the light and heavy groups. The similarity of the two groups in this last respect agrees with the insignificant correlation between dressed weight and frequency of follicles 1–3 mm in diameter and frequency of blood follicles (fig. 1).

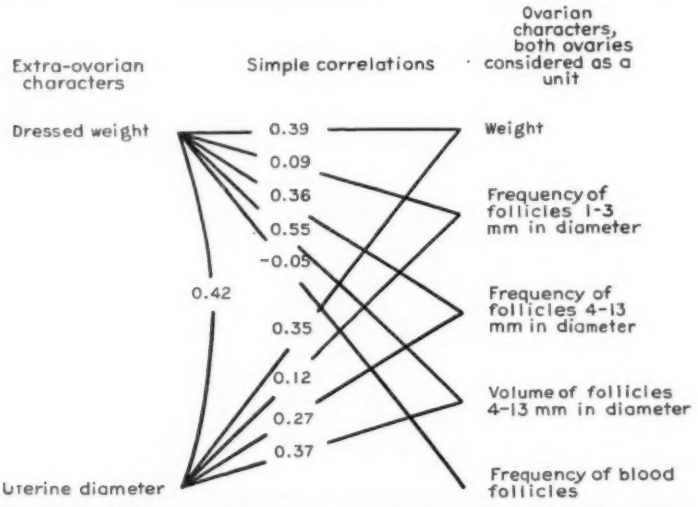

FIGURE 1.—Association among ovarian and extra-ovarian characters in 190 calves of Holstein appearance. Significant correlation=0.15; highly significant=0.18.

TABLE 2.—*Ovarian characters as related to dressed weight of calves of Holstein appearance*

Calves having—	Percentage of calves of indicated dressed weight having character described	
	91 calves of 52 to 86 pounds	99 calves of 87 to 138 pounds
Visible follicles (any size)	97.80	98.99
Follicles over 3 mm in diameter	80.22	96.97
Follicles over 6 mm in diameter	61.53	87.88
Follicles over 9 mm in diameter	25.26	54.54
Follicles 4 to 6, 7 to 9, and 10 to 13 mm in diameter	7.69	28.28
More than 1 follicle over 9 mm in diameter	0.00	8.08
More than 50 follicles less than 4 mm in diameter	24.20	22.22
Blood follicles	50.54	48.48

The diameter of the uterine horn was used as a measure of the amount of stimulation being exerted upon the uterus. The significant association between uterine diameter and dressed weight (fig. 1) is interpreted as being, in large part, due to ovarian development, which itself was highly correlated with dressed weight. Significant correlations were obtained between uterine diameter and ovarian weight, and number and volume of follicles 4–13 mm in diameter. Inasmuch as follicular hormone is an important factor in uterine development these correlations suggest that the follicular apparatus in the immature calf is increasing with age not only in magnitude but also in physiological activity, as shown by growth and development in the genital tract.

A comparison was made next of the various ovarian characters in the right and left ovaries respectively. While the situation in the two breed groups of calves was not identical, in the main it was very similar. In the Holsteins consistently higher values were found for the various characters of the right ovaries than for those of the left ovaries with the exception of frequency of blood follicles (table 3). In the calves of other breeds the same was true for the weight and dimensions of the ovary and for the volume of follicles 7–13 mm in diameter. The mean total follicular volume of follicles 4–13 mm in diameter is significantly greater in the right ovaries of both groups, and the mean weight of the right ovary was greater than that of the left in the Holsteins.[8]

TABLE 3.—*Comparison between dimensions and character of right and left ovaries of calves of Holstein appearance and calves of other breeds*

Item	Holstein		Other breeds	
	Right $M \pm \sigma_M$	Left $M \pm \sigma_M$	Right $M \pm \sigma_M$	Left $M \pm \sigma_M$
Ovaries, number	190	190	83	83
Dimensions:				
Length, millimeters	20.08 ± 0.28	19.42 ± 0.30	20.66 ±0.44	19.71 ± 0.43
Width, do	9.75 ± .23	9.22 ± .21	10.31 ± .32	10.23 ± .36
Depth, do	6.83 ± .18	6.55 ± .16	7.47 ± .25	7.40 ± .19
Weight, grams	1.01 ± .05	.89 ± .04	1.12 ± .08	1.03 ± .08
Frequency of follicles of different diameters:				
1 to 3 mm	18.9 ± 1.9	17.9 ± 1.8	16.7 ± 2.4	18.6 ± 2.7
4 to 6 mm	1.79 ± .18	1.55 ± .16	1.81 ± .21	2.18 ± .28
7 to 9 mm	.57 ± .07	.50 ± .06	.74 ± .08	.55 ± .11
10 to 13 mm	.28 ± .03	.17 ± .03	.34 ± .06	.22 ± .05
4 to 13 mm	2.64 ± .19	2.22 ± .17	2.88 ± .24	2.95 ± .29
Volume of follicles of different diameters:				
4 to 6 mm, cubic millimeters	104 ± 10	88 ± 9	111 ± 12	122 ± 14
7 to 9 mm, do	148 ± 19	118 ± 14	184 ± 22	131 ± 25
10 to 13 mm, do	194 ± 24	116 ± 20	208 ± 36	131 ± 29
4 to 13 mm, do	447 ± 32	317 ± 25	504 ± 43	384 ± 40
Frequency of blood follicles	.49 ± .07	.55 ± .08	.69 ±.18	.65 ± .16

Significant correlations were found between the right and left ovaries of the Holstein calves for the following characters: Frequency of blood follicles, frequency of follicles 4–13 mm in diameter, weight of the ovary, and frequency of follicles 1–3 mm in diameter (table 4). Although the two ovaries showed association in each of these characters, there were significant differences in the values of the correlation coefficients; the items are listed in the preceding sentence in the

[8] The curves for the distribution of each of the ovarian characters are asymmetrical. Although the type of asymmetry is similar and N is the same for the right and left ovaries, there may be some question as to the validity of this test for significance of the difference. Corroboration of all the differences between the right and left noted in the text was obtained by use of the point binomial, which showed a significantly excessive occurrence of greater values for the characters in the right than in the left ovary.

ascending order of their importance. The volume of follicles 4–13 mm in diameter showed no correlation of significance between the right and left ovaries.

Tables 5 and 6 are presented for the purpose of placing on record the data on normal variation in ovaries of calves of veal size and normal values of ovarian characters for calves of different weights. In addition, they show the reasonably consistent difference between the mean values of the ovarian characters in the right and left ovaries of the Holstein calves when divided into classes based on dressed weight of the calf and the combined weight of the two ovaries.

TABLE 4.—*Comparison of the weight and characters of the right and left ovaries in 190 calves of Holstein appearance*

Ovarian character	Right [1] ovary	Left [1] ovary	Relation between right and left ovary	
			Probability of mean difference	Simple correlation
Weight.....gram	1.01	0.89	<0.01	0.74
Frequency of follicles 1 to 3 mm in diameter	18.9	17.9	.15–.16	.93
Frequency of follicles 4 to 13 mm in diameter	2.64	2.22	.08–.09	.60
Volume of follicles 4 to 13 mm in diameter...cubic millimeter	447	317	<.01	.05
Frequency of blood follicles	.49	.55	.40–.50	.37

[1] Refer to table 3 for σ_M of ovarian characters.

TABLE 5.—*Weight and characters of right and left ovaries of calves of Holstein appearance as related to dressed weight of calf*

Dressed weight (pounds)	Calves	Weight of—		Frequency of follicles 4 to 13 mm in diameter		Volume of follicles 4 to 13 mm in diameter,	
		Right ovary $M\pm\sigma_M$	Left ovary $M\pm\sigma_M$	Right ovary $M\pm\sigma_M$	Left ovary $M\pm\sigma_M$	Right ovary $M\pm\sigma_M$	Left ovary $M\pm\sigma_M$
	Number	*Grams*	*Grams*	*Number*	*Number*	*Cubic millimeters*	*Cubic millimeters*
52 to 72	27	0.65±0.09	0.53±0.08	1.5±0.3	1.0±0.2	237± 61	87± 24
73 to 80	37	.80± .11	.81± .08	2.1± .4	1.7± .3	303± 81	271± 48
81 to 88	39	.95± .10	.80± .08	2.4± .3	1.7± .3	401± 55	254± 50
89 to 96	31	1.05± .09	.90± .09	2.8± .4	2.7± .4	491± 59	336± 66
97 to 104	22	1.07± .14	1.10± .15	3.3± .9	2.7± .6	480± 89	573± 94
105 to 112	23	1.52± .17	1.23± .19	3.6± .5	3.5± .6	687± 35	369± 44
113 to 138	11	1.51± .16	1.20± .20	4.4± .7	4.0± .8	918±130	595±150

TABLE 6.—*Weight and characters of right and left ovaries of calves of Holstein appearance as related to weight of the two ovaries as a unit*

Weight of both ovaries (grams)	Calves	Weight of—		Frequency of follicles 1–3 mm in diameter		Frequency of follicles 4–13 mm in diameter		Volume of follicles 4–13 mm in diameter	
		Right ovary $M\pm\sigma_M$	Left ovary $M\pm\sigma_M$	Right ovary $M\pm\sigma_M$	Left ovary $M\pm\sigma_M$	Right ovary $M\pm\sigma_M$	Left ovary $M\pm\sigma_M$	Right ovary $M\pm\sigma_M$	Left ovary $M\pm\sigma_M$
	Number	*Grams*	*Grams*	*Number*	*Number*	*Number*	*Number*	*Cubic millimeters*	*Cubic millimeters*
0.2 to 0.7	25	0.29±0.02	0.26±0.02	3.2±1.1	2.8±1.0	0.5±0.2	0.4±0.1	43± 16	26±10
0.8 to 1.3	39	.52± .03	.51± .03	7.0±1.3	7.2±1.6	1.2± .2	1.5± .3	243± 47	243±41
1.4 to 1.9	62	.86± .04	.81± .04	12.1± .5	9.4±1.0	2.8± .3	2.0± .2	504± 57	399±47
2 to 2.5	24	1.27± .06	.93± .05	14.3±2.2	14.0±2.4	3.6± .5	2.8± .4	741± 92	403±78
2.6 to 3.2	19	1.72± .05	1.23± .08	37.5±5.6	36.7±5.4	4.5± .6	3.1± .8	758±106	328±94
3.3 to 6.4	21	2.30± .11	2.22± .12	68.6±7.7	68.2±7.2	4.6±1.0	4.5± .8	521± 80	452±74

DISCUSSION

The data presented in this study show that follicular development in the calf ovary is well under way a few days after birth and in general is augmented with age. This suggests that there is an increasing gonad-stimulating activity on the part of the anterior pituitary long before the time of puberty. The significant correlation between total volume of larger follicles and diameter of the uterine horn further suggests that follicular development in the ovary is not merely a morphological phenomenon but that the follicles in these sexually immature calves are to a degree physiologically active and producing an effect in uterine development. The trend of ovarian development demonstrated by this study appears to substantiate the idea that the morphological state and physiological activity of the ovary which are attendant upon puberty are the product of a gradual development, and are not effected briefly before reproductive maturity is attained.

On the basis of the mere presence of vesicular follicles, it would seem likely that almost all dairy calves of veal age should react experimentally to gonad-stimulating hormones. The minimum size of the vesicular follicle which is necessary to give the reaction is not determined by this study; but, considering the comparatively large size of the follicles in most calves, probably the majority of them would react to pituitary extracts.

The normal variability in calf ovaries indicates the necessity of determining the condition in each animal before experimental treatment. Especially is this true if the change to be produced is expected to be small. The normal values of the different characters reported in this study may be helpful in determining the degree of change produced experimentally in terms of normal change occurring with increasing body and ovarian weights.

The presence of follicles of ovulation or immediately preovulation size which do not rupture emphasizes the need for determination of the estrin content of follicles of different sizes and also the conditions in the mucosa and musculature of the genital tracts which are associated with them. The increased diameter of the uterine horn may indicate the functional activity of the ovary with regard to increasing secretion of estrin, but at what stage of its development the follicle begins to produce a physiological effect on the genital tract is unknown.

It seems unlikely that the large follicles which are present in the young calf persist in the ovary until puberty. While in general the number and size of large follicles appear to increase with the age of the animal, much variation occurred throughout the series and an appreciable number of calves in the younger group showed greater follicular development than some calves in the older group. It could not be determined in this study whether there were periodic waves of follicular growth in the ovaries as a whole, but it would seem that individual follicles develop and regress somewhat independently of other follicles in the same animal. The consistent occurrence of blood follicles in all groups of calves suggests that a rather continuous follicular degeneration takes place during the first few months of postnatal life. The number of enlarging follicles present at any one time, however, tends to increase as the animal grows older.

SUMMARY

Ovarian and follicular development and their relation to body weight and uterine size have been studied in 273 dairy calves, ranging from a few days to approximately 14 weeks of age as estimated from dressed weight. Vesicular follicles were present in practically all animals. Ovarian weight and degree of development of larger follicles were significantly correlated with both body weight and uterine diameter. Although a high degree of association existed between the right and left ovary with respect to weight and number of follicles, there were significant differences between the mean weight and the total follicular volume of follicles 4–13 mm in diameter in the two ovaries, the right having the greater values. Approximately one-half of the calves (irrespective of weight) had blood follicles present in their ovaries, suggesting continuous follicular degeneration during early postnatal life.

EFFECT OF FERTILIZERS, SOIL TYPE, AND CERTAIN CLIMATIC FACTORS ON THE YIELD AND COMPOSITION OF OATS AND VETCH [1]

By G. O. Baker and S. C. Vandecaveye,[2] *Washington Agricultural Experiment Station*

INTRODUCTION

Climatic conditions as well as soil type and fertilizers may be expected to influence the yield and composition of crops. Although much work has been done to determine the effect of fertilizers and soil types on the yield and composition of crops, very little information is available on the influence of climatic factors when the same soil or culture solution is used under different climatic conditions the same year. The conclusions of Dickson (*6*) [3] that oats grown at Madison, Wis., contained a higher percentage of calcium and phosphorus than oats grown at Pullman, Wash., under the same cultural conditions were not based on work done the same year but in different years. Variations in climatic conditions in the same locality from year to year may also be expected to result in differences in composition of crops. Likewise the report of Delwiche and Tottingham (*5*) to the effect that the nitrogen content of corn, barley, and clover was only slightly influenced by climatic conditions is open to question as these crops were grown on two different soil types, which by their very nature may cause differences in the composition of crops. It is apparent, therefore, that while the effect of fertilizers on crop yield is generally conceded, any differences in composition that may result from applications of fertilizers, differences in soil type, and variations in climate can be determined with certainty only when the same soils receiving identical treatments are subjected to different climatic environments the same year.

In an attempt to obtain some light on this subject a series of pail experiments with three widely different soil types was started in 1931. The experiments conducted at Pullman were duplicated in southwestern Washington in the area from which these three soils were obtained. In this manner the same soil receiving identical cultural treatments was subjected to two different climatic environments, the humid climate of southwestern Washington and the semiarid climate of eastern Washington.

The climate of southwestern Washington is of the marine type, having a narrow annual temperature range, an annual mean temperature higher than the average for the latitude, and heavy precipitation with a maximum in the winter months. Associated with the high rainfall is a high relative humidity and a large percentage of cloudy or partly cloudy days which may be expected to result in a

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[2] The authors gratefully acknowledge the assistance received during the progress of this work. Thanks are due the late H. F. Holtz for suggesting and aiding in outlining the program; R. E. Bell, formerly instructor in soils, State College of Washington, for help in getting the work started; Dr. H. F. Clements, associate professor of botany, State College of Washington, for reviewing the manuscript; and H. S. Porter and B. L. Brown, Smith-Hughes instructors at Chehalis and Woodland, respectively, for their splended cooperation in taking care of the experiments located in southwestern Washington.

[3] Reference is made by number (italic) to Literature Cited, p. 974.

low-light intensity. The elevation of this area is relatively low; that of the city of Centralia, which is centrally located in the experimental area, is only 182 feet above sea level. The climate of eastern Washington is essentially continental in character, having a wide annual temperature range. The precipitation is light and occurs largely in the winter period. The relative humidity is low, the number of clear days large, and the light intensity high. The elevation of this area ranges from 1,000 to 2,700 feet, that of the city of Pullman being 2,550 feet.

EXPERIMENTAL PROCEDURE

The three soil types selected for this experiment were (1) Felida silt loam from Clarke County, a soil derived from ancient alluvial or marine sediments; (2) Olympic loam from Cowlitz County, a soil derived from residual material; and (3) Chehalis clay loam from Lewis County, a soil derived from alluvial material. The normal annual precipitation in these three areas is approximately 37, 63, and 45 inches, respectively.

The samples of soil were obtained from near the check plots of experimental fields previously established by the Washington Agricultural Experiment Station. Soil taken from the upper 8 inches was thoroughly mixed, half of the amount sent to Pullman, and the other half retained by the cooperators in southwestern Washington. Each sample was sufficient to fill, in each of the two locations, 12 galvanized pails of 12-quart capacity to a depth of 8 inches. The pails were painted with asphaltum paint and provided with drains. Six pails were planted to oats (*Avena sativa* L.) and six to vetch (*Vicia sativa* L.). Each of the two sets of experiments received the following treatments: Check, N, NP, NPCa, NPKCa, and NCa.

Since the primary interest was in the mineral content of the crops, nitrogen was added to all pails except the check so as to eliminate the possibility of its being a limiting factor in plant growth. Chemically pure chemicals were used to avoid so far as possible the complicating effect of certain elements that might otherwise have been introduced. The diameter of the pails, 4 inches from the bottom, served as a means of calculating the area on which to base the rate of fertilizer application. The chemicals applied and their equivalents in fertilizers calculated on the acre basis are given in table 1.

TABLE 1.—*Chemicals applied to pails of soil and their equivalents in fertilizers calculated on the acre basis*

Treatment	Materials added	Fertilizer equivalent [1]	Approximate rate per acre
Check:			*Pounds*
N	$NaNO_3$	Sodium nitrate	300
NP	$NaNO_3$	do	300
	$NaH_2PO_4.4H_2O$	Superphosphate	600
NPCa	$Ca(NO_3)_2.4H_2O$	Sodium nitrate	300
	$CaH_4(PO_4)_2.H_2O$	Superphosphate	1,200
	$CaCO_3$	Lime	4,000
NPKCa	$NaNO_3$	Sodium nitrate	300
	KNO_3	Muriate of potash	270
	$CaH_4(PO_4)_2.H_2O$	Superphosphate	1,200
	$CaCO_3$	Lime	4,000
NCa	$Ca(NO_3)_2.4H_2O$	Sodium nitrate	300
	$CaCO_3$	Lime	4,000

[1] Sodium nitrate, 15.5 percent N; superphosphate, 17 percent P_2O_5; muriate of potash, 48 percent K_2O; lime, 100 percent $CaCO_3$.

The chemicals were thoroughly mixed with the soil and sufficient water was added to bring the moisture content to normal field capacity as expressed by Shaw (*14*). The pails were covered and allowed to stand 3 or 4 days before seeding. Following the harvest, the soils were allowed to dry and were stored under cover during the winter. The second year chemicals were again mixed with the soil as in the previous year, water was added, and the moist soils were allowed to stand a week before seeding.

The three soils studied in southwest Washington, referred to as "the coast", were located at Chehalis in 1931, as were the Chehalis and the Felida soils in 1932. The Olympic soil was located at Woodland in 1932. The work in these localities was done by two cooperators and was conducted in the same manner as at Pullman insofar as it was possible to do this under cooperative arrangement.

Swedish Select oats and common vetch, grown on the college farm at Pullman, were used as seed. The vetch seed was inoculated with a culture of the proper legume bacteria. In both localities the oats and vetch were planted the same day at the rate of 20 seeds per pail. After the seedlings were well established they were thinned to 10 plants.

Each pail was weighed at the outset and the plants were watered frequently (every day during the latter part of the growing season). The pails were made up to weight once a week if necessary by the addition of water. Condensed steam served as a source of water at Pullman. Since neither condensed steam nor distilled water was available at Chehalis and Woodland, the regular city water was used. Although the water in these areas is known to have an extremely low mineral content, a chemical analysis revealed that it contained 7.5 parts per million of calcium, the chief element in water that might influence the results.

Both oats and vetch were harvested simultaneously, for any one soil, at a time when most of the oat plants were in the soft dough stage. The crops on the coast matured a week to 10 days later than at Pullman. After harvest, the crop samples were oven-dried at 65° C., weighed, and ground for chemical analysis.

Nitrogen was determined by the official Kjeldahl method (*2*).

For the determination of the mineral elements the oven-dried plant material was ashed in a muffle furnace at a dull red heat. The ash was taken up with 1:4 hydrochloric acid, diluted with hot water, and filtered into volumetric flasks. Phosphorus was determined by the official method (*2*) with slight modifications, calcium by Chapman's method (*4*), and potassium by the sodium cobaltinitrite method of Volk and Truog (*15*). In all cases the composition is reported in terms of percentages based on oven-dry weight of the plant material.

CLIMATIC CONDITIONS

The available weather data for the coast area are not as complete as those for Pullman, but the United States Weather Bureau data for Centralia, which is 4 miles distant from Chehalis and located in the same river valley, are considered fairly representative and are the best that are available for this purpose. The weather data for the growing seasons of 1931 and 1932 for both localities are given in table 2.

It is noted that the mean minimum temperature for the months of May, June, July, and August in both 1931 and 1932 was lower at Centralia than at Pullman. The mean maximum temperature at Centralia was higher during May and June and lower during July and August than at Pullman. Thus it is evident that the daily temperature differences were greater on the coast, especially during May and June.

In 1932 Pullman experienced a much greater number of clear days than Centralia and less than one-third as many cloudy days. In 1931 the difference in the character of the day for the two localities, although appreciable, was not as great.

Pullman received only 1.81 and 1.84 inches of precipitation during the growing seasons of 1931 and 1932 respectively, as compared to 5.13 and 3.35 inches for Centralia. Since the plants were watered regularly, they were not dependent on the rain for their moisture; but in all probability the difference in the precipitation and number of clear days had a direct effect on plant transpiration and the relative humidity of the air.

TABLE 2.—*Climatic data for Pullman and Centralia, Wash., from May 10 to August 10, 1931 and 1932*

Year and month	Mean temperature		Character of day			Days when precipitation was—		Precipitation
	Maximum	Minimum	Clear	Partly cloudy	Cloudy	Less than 0.2 inch [1]	Over 0.2 inch	
	PULLMAN							
1931:	°F.	°F.	Number	Number	Number	Number	Number	Inches
May	69.4	46.5	10	8	3	1	1	0.44
June	70.2	50.5	12	8	10	5	3	1.37
July	83.4	54.6	20	9	2	0	0	.00
August	82.0	54.4	10	0	0	0	0	.00
1932:								
May	63.9	43.9	12	5	4	5	2	1.29
June	74.8	51.3	20	6	4	0	1	.24
July	78.8	53.4	21	9	1	3	1	.31
August	83.7	59.1	8	2	0	1	0	.00
	CENTRALIA							
1931:								
May	74.3	43.2	10	6	5	4	2	0.86
June	71.6	47.8	7	8	15	11	9	4.18
July	81.1	51.3	24	5	2	2	0	.08
August	81.3	50.4	7	3	0	1	0	.01
1932:								
May	66.6	42.5	8	5	8	9	1	.58
June	76.0	47.5	18	6	6	5	1	.37
July	72.6	49.7	12	5	14	8	3	2.16
August	79.6	52.1	7	0	3	4	0	.24

[1] Includes days designated as trace.

Because of the heavy rainfall at Centralia in June of 1931, the total exceeding 4 inches, water stood in the pails for a day or two after heavy rains and temporary waterlogging resulted. This may have had a slight effect upon the development of the crops.

CROP YIELDS

EFFECT OF FERTILIZER TREATMENT AND SOIL TYPE

It is often observed that the application of the same fertilizers will not always give the same crop response under apparently similar conditions, perhaps because environmental factors other than fertilizers exert some influence.

The individual crop yields as a result of different fertilizer treatments, soil type, and climatic conditions showed extreme variation. Those of oats ranged from 10.0 to 77.5 g and those of vetch from 1.5 to 46.4 g per pail. Considering the average yields of the 2 years for each of the 2 crops as influenced by fertilizer additions alone, and disregarding soil type and climate, as shown in figure 1, it is evident that the 2 crops responded very differently to fertilizer treatments. The addition of nitrogen alone resulted in a marked increase in yield of oats but depressed the yield of vetch. When phosphate was added in addition to nitrogen, both crops responded about equally in yield, as compared to the nitrogen treatment. There is no clear-cut evidence that the doubling of the amount of phosphate fertilizer contributed materially to crop yields since the apparent increase in yield of vetch resulting from the NPCa treatment, which contained twice as much phosphate as the NP treatment, may have been caused by the addition of lime.

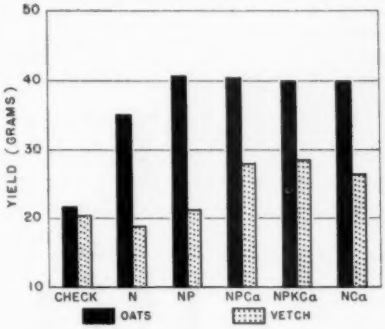

FIGURE 1.—Effect of fertilizers on yields of oats and vetch (average of all experiments).

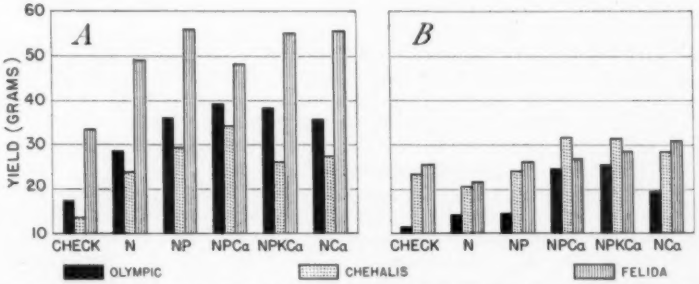

FIGURE 2.—Effect of soil type on yields of oats (*A*) and vetch (*B*) (average of 2 years and both locations).

When lime was used in combination with other fertilizer elements, the oat yields were not materially affected, but the vetch yields were materially increased.

Potassium fertilizer in combination with the other fertilizers had very little effect on yield.

When soil types alone are compared, as in figure 2, it is evident that the same general effects are indicated but with greater or less

variation. Of the three soils studied, Felida demonstrated the greatest natural productivity and also produced the greatest yields as a result of fertilizer additions, except that the NPCa and the NPKCa treatments when used for vetch gave slightly better results on the Chehalis soil.

The Olympic soil produced the lowest average yield of vetch. Vetch grown on this soil at Pullman the second year gave exceedingly low yields in the absence of lime—an average of 3.5 g—and high yields in the presence of lime—an average of 38.3 g. Since this condition was observed only at Pullman, it was decided to repeat the experiment with vetch on this soil the third year. The same relative results were obtained. This observed difference in response between the two locations may be partially due to the small amount of calcium present in the water used on the coast.

EFFECT OF CLIMATE

Although certain tendencies in the yield of oats and vetch appear to be characteristic effects of fertilizer treatments and soil types, climatic variations may also be expected to modify these results.

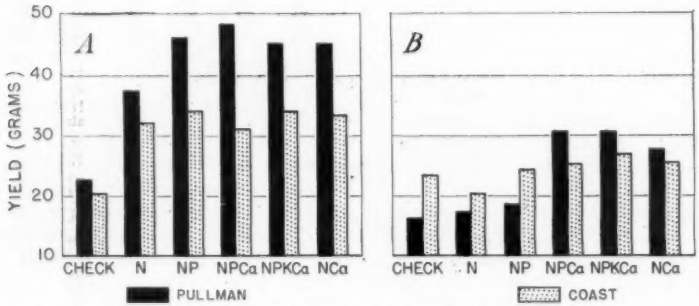

FIGURE 3.—Effect of local climate on yields of oats (*A*) and vetch (*B*) (average for all soils for 2 years)

The average yield for all soils for 2 years for each crop in each location is shown in figure 3. It is observed that oats yielded more at Pullman than on the coast, especially in the soils treated with P, K, and Ca in various combinations. Vetch in the presence of lime also yielded more at Pullman than on the coast. However, in the absence of lime higher yields of vetch were obtained on the coast.

The climatic effect is not necessarily limited to different localities but may exert its influence in the same locality in different years, as has been pointed out by Caldwell (*3*) and as can be seen from an analysis of the data presented by Delwiche and Tottingham (*5*), Dickson (*6*), and Frankena (*10*). Similar results are indicated in figure 4, where the average yields of oats and vetch for the three soil types are illustrated for 1931 and 1932.

The yield of oats was greater at Pullman than on the coast in 1931 but the reverse was true the following year except for the NPCa treatment. The difference in the yield of vetch between the two localities was not as great as for oats the first year, although in general the better yields were obtained at Pullman. In 1932, vetch in the

absence of lime gave considerably greater yields on the coast but when lime was present, as already explained, the yields at Pullman were greater. This difference in response the second year was probably due to an actual shortage of calcium as a nutrient, and as a result of a greater total removal of calcium at Pullman this deficiency appeared there first.

The effectiveness of any particular fertilizer, as indicated by yields, may vary with the different seasons, as is shown in figure 4. In 1931 in both locations the addition of nitrogen to vetch increased the yield, whereas in 1932 the yield was reduced when nitrogen was added. This lack of response the second year may have been due to better inoculation.

When phosphate was supplied in addition to nitrogen, the yield of vetch grown on the coast was reduced in 1931 and increased in 1932, while at Pullman the reverse was true.

At Pullman the addition of lime reduced the oat yields in 1931 and increased them in 1932; it was only slightly beneficial for vetch in 1931 but greatly increased the yield in 1932. On the coast the addition of lime had very little influence on the yield of either oats or

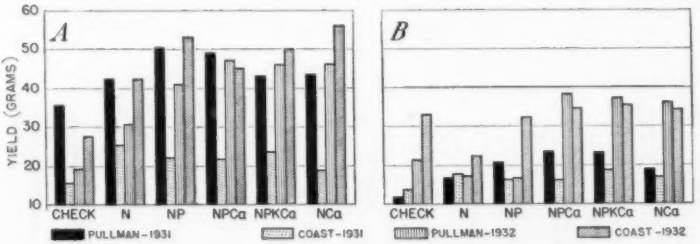

FIGURE 4.—Effect of local climate in different years on the yield of oats (*A*) and vetch (*B*) (average for all soils), 1931 and 1932.

vetch in 1931 or 1932. The small amount of lime supplied by the water used may partially account for the lack of response.

A careful analysis of the data on yields reveals certain outstanding facts. The results clearly indicate that in evaluating the effect of fertilizers on crop yields, the kind of crop, the soil type, and the climatic environment must be considered as well as the kind and amount of fertilizers applied. Although suitable quantities of the proper combination of fertilizers resulted in a generous crop response in all cases, the average increase in yield for the 2 seasons on the 3 soils irrespective of location was approximately 87 percent for oats and only about 42 percent for vetch. As for the influence brought about by soil type disregarding climatic environment, the extremes in the average increase in yield for oats for the 2 seasons varied from 155 percent for the Chehalis soil to 67 percent for the Felida soil, and for vetch from 126 percent for the Olympic soil to 21 percent for the Felida soil.

It is a common experience in field experiments to find that repeated applications of the same fertilizers to the same soil do not give the same results from year to year. Variations in available moisture and especially a deficiency of moisture are generally accepted as the major

responsible factors. Since these factors were entirely eliminated in these experiments by providing an adequate supply of available moisture for the crops at all times, other climatic factors undoubtedly had an important bearing on the crop growth. Arthur (*1*) pointed out that light intensity and light quality as well as other climatic factors and soil conditions affect plant growth. When the yields of the 3 soils for the 2 seasons are averaged, the results show that the most favorable fertilizer treatment resulted in a 111 percent increase in the yield of oats at Pullman and only an 89 percent increase on the coast. The yield of vetch, when calculated on the same basis, returned a 67 percent increase at Pullman and only a 13 percent increase on the coast.

CHEMICAL COMPOSITION

EFFECT OF FERTILIZER TREATMENT AND SOIL TYPE

The effect of fertilizers on the composition of crops is somewhat of a controversial matter, perhaps because environmental factors other than fertilizers exert their influence. Gericke (*11*) suggests that some of the unexplained discrepancies that commonly are encountered in comparative studies on plant salt requirements and the application of fertilizer to agricultural soils may be related to climatic influences.

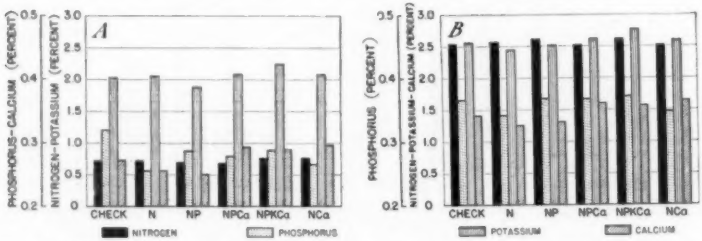

FIGURE 5.—Effect of fertilizers on the chemical composition of oats (*A*) and vetch (*B*) (average for all soils for 2 years and both locations).

Considering the effect of fertilizers from the standpoint of crops alone, and disregarding soil type and climate as is indicated in figure 5, the average composition of oats and vetch grown during two different seasons was most affected by the additions of phosphate fertilizers and lime. When a phosphate fertilizer was added to the soil, the percentage of phosphorus in both oats and vetch was increased above that in oats and vetch which received fertilizer other than phosphate but not above that in the unfertilized plants. The addition of lime to the soils caused an increased percentage of calcium in both oats and vetch above that of plants grown on soils which received no lime. The presence of lime also slightly increased the potassium content of both oats and vetch. The addition of nitrogen alone definitely reduced the percentage of phosphorus and calcium in both oats and vetch. This tendency seemed to be apparent in regard to the phosphorus content of oats even when nitrogen was used in combination with the other fertilizers. This may be partially accounted for by increased yields resulting from the nitrogen fertilizer, since the oats were considerably more affected in this respect than the vetch.

When soil types alone are considered, however, it is evident that although the same general effect is indicated the different soils show considerable variation, especially in the phosphorus and calcium content of both crops. This is shown in figure 6. Oats and vetch grown on Olympic soil, in general had a lower phosphorus and calcium content than those grown on the other soils. The application of phosphate to the Olympic soil did not generally increase the phosphorus content of these crops, but when applied to the Felida and

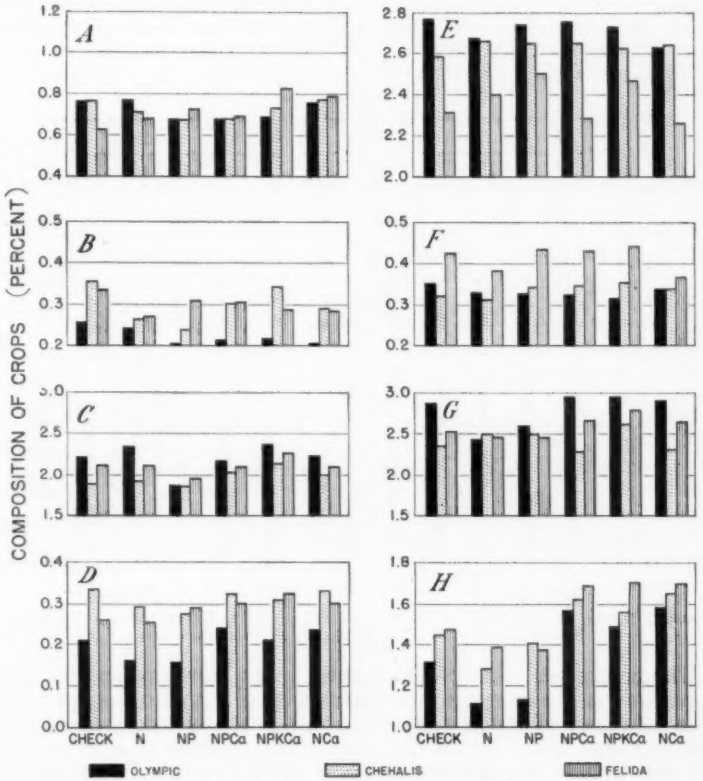

FIGURE 6.—Effect of soil type on the chemical composition of oats and vetch (average for 2 years and both locations): *A* to *D*, Oats; *E* to *H*, vetch; *A* and *E*, nitrogen content of crop; *B* and *F*, phosphorus content of crop; *C* and *G*, potassium content of crop; *D* and *H*, calcium content of crop.

Chehalis soils, it resulted in a uniform rise of the phosphorus content of the vetch but not of the oats. The addition of lime markedly increased the calcium content of oats on the Olympic soil and only slightly on the other soils. The influence of soil type was less apparent in regard to the calcium content of vetch when lime was used, but again the greatest effect was obtained on the Olympic soil. Fonder (*7*, *8*, *9*) also found a considerable variation in the magnesium as well as in the calcium content of alfalfa, beans, and peas when grown on

different soil types under the same general climatic conditions in the same locality the same year. The general depressing effect of nitrogen fertilizer on the phosphorus content of the crops, although greater on the Olympic soil, was never completely overcome on either the Chehalis or the Felida soil by any of the fertilizer combinations regardless of the relatively large amounts of phosphate fertilizers used in some of the treatments.

The nitrogen and potassium content of the crops was much less affected by soil type. The results for nitrogen are in accord with those obtained by Delwiche and Tottingham (*5*) and Le Clerc (*12*), who found that the percentage of nitrogen in the crops they studied was influenced only slightly by soil type even though great variation in types existed.

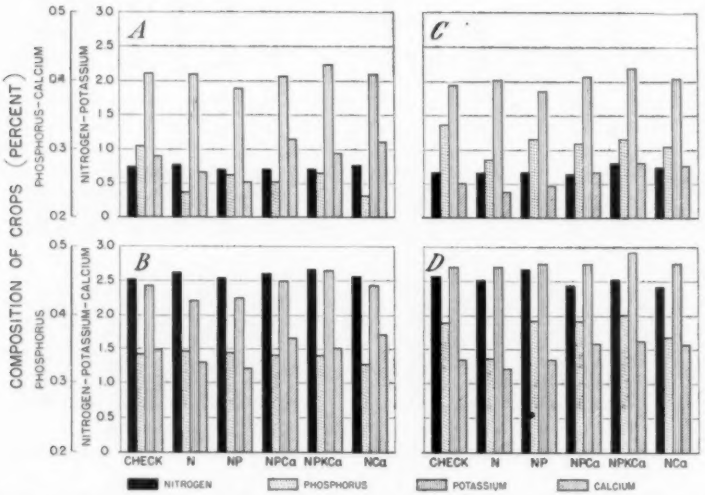

FIGURE 7.—Effect of local climate at Pullman (*A*, *B*) or on the coast (*C*, *D*) on the chemical composition of oats (*A*, *C*) and vetch (*B*, *D*) (average for all the soils for 2 years).

The percentage of a specific element in a plant may be somewhat dependent on the interaction of the soil with the specific element added as a fertilizer. For example, when phosphate in combination with nitrogen was added to the Olympic soil, it resulted in a decrease in the phosphorus percentage of both oats and vetch below that of nitrogen alone but when added to Felida an increase in phosphorus content was observed.

EFFECT OF LOCAL CLIMATE

Although certain definite tendencies in the composition of oats and vetch appear to be characteristic effects of fertilizer treatments and soil types, these are undoubtedly modified by climatic influences. This was very strikingly shown when the average composition of the two crops was considered, as indicated in figure 7. It was found that the percentage of phosphorus was higher and the percentage of calcium

was lower on the coast than at Pullman. This phosphorus-calcium-climate relationship has been observed also in unpublished work done in this laboratory when the composition of alfalfa grown on the coast was compared with that of alfalfa grown in the irrigated valleys of the central part of the State. This cannot be entirely attributed to annual climatic changes, as the same general comparison was observed in 1931 and in 1932.

The data in table 2 show that the total number of clear days was greater and the precipitation lower at Pullman than on the coast for both years. This fact, which corresponds with the results of MacMillian's work (*13*) in which he concluded that localities with higher elevation and lower relative humidities receive a greater total amount of ultraviolet rays, indicates that the amount of ultraviolet light received at Pullman should be greater than that received on the coast for both years. Since Wynd and Fuller (*16*) found that tomato and cucumber plants subjected to ultraviolet radiation contained a higher percentage of calcium and a lower percentage of phosphorus than unradiated plants, it seems that the amount of ultraviolet radiation may be one of the important factors that determined the observed phosphorus-calcium relation between the two different localities.

Applications of lime were more effective in increasing the calcium content of both oats and vetch at Pullman than on the coast. The calcium content of the water used on the coast did not have any appreciable effect on the observed results if the chemical composition of the oats and vetch grown is used as a criterion, for in all cases except the NP and NPKCa treatments of the latter crop the calcium content was lower on the coast.

There was very little difference in the nitrogen and potassium content of the oats grown in the two localities, while for vetch the potassium content was higher on the coast and the nitrogen content higher at Pullman.

The climatic effect was not limited to different locations but showed its influence in the same localities in different years. This was also shown in the work of Caldwell (*3*), Frankena (*10*), and Dickson (*6*). The results are illustrated in figure 8 in which the composition of the same crop grown in the same locality is graphed separately for each year.

The percentage of nitrogen in vetch was higher in 1932 than in 1931 for both localities, while in oats the nitrogen content was higher at Pullman in 1931 than in 1932 and on the coast no consistent results were observed. The phosphorus and potassium content of both oats and vetch, on the coast and at Pullman alike, was more or less greater in 1931 than in 1932, but the calcium content was not uniformly affected by seasonal climatic variations in either locality.

The existing differences in the mean daily temperature, in condition of the day, and in precipitation in the same locality in different years did not seem to exert any appreciable effect on composition. It is possible that other climatic factors may be the dominant ones as far as climate is concerned when composition changes are produced, or the cause may be a balance between all climatic factors.

In recent years considerable significance has been attached to the phosphorus and calcium content of feeds for livestock. It has been found that livestock fed on pasturage and hay containing these

elements in amounts below a certain critical figure suffer from nutritional diseases. The many controversial data presented in the literature on the effect of fertilizers on the composition of crops cast doubt upon the practicability of improving the mineral content of forage crops by means of fertilizer applications to the soil. However, in the large majority of these cases no consideration has been given to the influence of soil type and climatic factors, both of which have clearly demonstrated their influence in the work here reported. Moreover, in many of the instances reported in the literature the phosphorus and calcium content of the crops grown without fertilizers

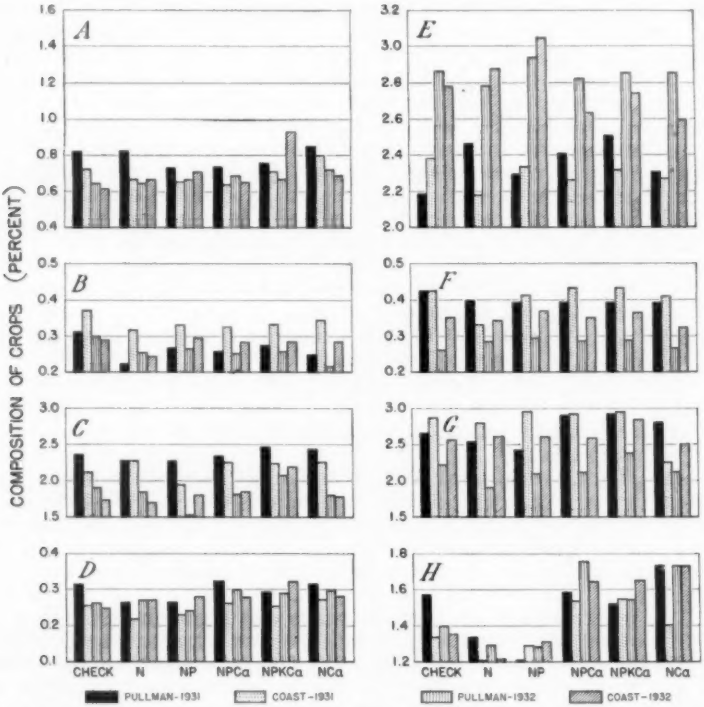

FIGURE 8.—Effect of the climate of different years on the chemical composition of oats and vetch (average of all soils), 1931 and 1932: *A* to *D*, Oats; *E* to *H*, vetch; *A* and *E*, nitrogen content of crop; *B* and *F*, phosphorus content of crop; *C* and *G*, potassium content of crop; *D* and *H*, calcium content of crop.

was not at the critical point, and consequently there was no urgent need for improvement of quality. The oats and vetch grown on the three soils employed here are typical examples. Nevertheless, applications of phosphates and lime to these soils counteracted the depressing effect of nitrogen on the phosphorus and calcium content of the crops, and although this beneficial influence was modified in varying degrees by soil type and climatic factors, it was general in all cases. If corresponding results can be obtained on soils that are so deficient in available phosphorus and calcium as to result in crops

with an abnormally low content of these elements, it seems that it should be possible to secure improved quality of crops as well as profitable returns when proper amounts of suitable fertilizers are applied to the soil.

SUMMARY AND CONCLUSIONS

Three of the more important soil types of southwestern Washington were selected for a series of pail experiments to study the effect of fertilizers, soil type, and local climate on the yield and chemical composition of oats and vetch. Duplicate sets of the same soil were used, one in the coastal climate of southwestern Washington in the ocality from which the soil was originally obtained and the other in the semiarid climate at Pullman. The results indicate that fertilizers, soil type, and local climate may have an important effect upon the yield and chemical composition of crops.

Fertilizer additions materially changed the yield of oats and vetch, the two crops responding differently to applications of nitrogen and lime and similarly to applications of phosphate and potassium. The chemical composition of the crops was likewise influenced by the use of fertilizers. Additions of nitrogen depressed the phosphorus and calcium content of both crops while additions of phosphate and lime increased the phosphorus and calcium content of the plants above that of the plants which received nitrogen fertilizers alone but not above that of the unfertilized plants.

The inherent soil characteristics, reflected in soil type, had a marked influence on the yield and chemical composition of the two crops. The same plant species showed a different relative response to the same fertilizers when grown on different types of soil. The percentage of calcium and phosphorus in the crops was especially affected by soil type, while the percentage of nitrogen and potassium was only slightly affected.

Climatic differences, excluding moisture, between the two localities also influenced the yield and chemical composition of oats and vetch. In general, the higher yields were obtained at Pullman. The same crops grown on the same soil under the same cultural conditions contained, on an average, a higher percentage of phosphorus and a lower percentage of calcium when grown in the coastal climate of southwestern Washington than they did when grown in the semiarid climate of Pullman. The effect of the addition of lime on the calcium content of both crops was more pronounced at Pullman than in southwestern Washington. The difference in the character of the light in the two localities probably resulted in a difference in the amount of ultra violet rays received by the plants, and this seemed to be related to the observed results in composition. It is evident that the dominant climatic factors involved are those other than temperature and precipitation.

The yield and chemical composition of the same crop on the same soil under the same cultural conditions may vary from year to year as a result of local climatic variations.

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PEAR ROOT CONCENTRATION IN RELATION TO SOIL-MOISTURE EXTRACTION IN HEAVY CLAY SOIL[1]

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INTRODUCTION

In the study of pear irrigation in the Rogue River Valley of Oregon it became important to determine those soil zones from which moisture was extracted most rapidly and also to learn whether such zones of greatest moisture loss corresponded to zones of greatest root concentration. The work carried on during 1931 and 1932 in heavy clay soil shows a clear-cut relation between observed root concentration and rate of soil-moisture extraction and provides some definite information as to the actual distribution and concentration of pear roots in this heavy clay soil.

SOIL-MOISTURE EXTRACTION BY ROOTS

METHOD OF MEASURING

The rate of soil-moisture extraction by the tree roots was measured by determining the decrease in soil-moisture content at different depths and at different distances from the trunk, during specific periods when no moisture was added to the soil. Soil samples were taken with the improved King tube in 1-foot increments from the under side of the mulch to bedrock, the depth of sampling never exceeding 8 feet. In the case of trees X–40 and MM–33, with no cultivation since the previous fall, there was no mulch, so sampling started at the surface. Standard methods were used in determining the moisture content of the samples. Obviously erroneous determinations were discarded.

TREES USED IN 1931

In the Fitch orchard one 24-year-old Bartlett tree (French roots), in a block planted 24 feet on the square, was used. This tree had a trunk circumference of 48.5 cm and a top volume of about 270 cubic feet. The soil is Meyer silty clay loam, 5 feet deep to a sandstone bedrock. The last irrigation for the season, on June 24, brought the soil moisture almost to the field capacity.

In the Klamath orchard one 26-year-old Anjou pear tree (French roots), in a block planted 26 feet on the square, was selected. This tree had a trunk circumference of 68.7 cm and an average limb spread of about 6.2 feet radially from the trunk. The soil is Meyer clay

[1] Received for publication Feb. 18, 1935; issued July 1935. Cooperative studies on irrigation of pears near Medford, Oreg., carried on by the U. S. Department of Agriculture and the Oregon Agricultural Experiment Station.

adobe, 4 feet deep to shale and marl bedrock. When this tree was irrigated for the last time, on July 16, the soil moisture was brought to 90 percent of the field capacity in the upper 4 feet.

Soil samples were taken along the four diagonals, the first sample 2 feet from the trunk and each succeeding sample 2 feet farther out, on the diagonal next in order, the outermost sample being 18 feet from the trunk. In addition, duplicates at 6 and at 12 feet from the trunk were taken on one side of each tree.

TREES USED IN 1932

Two large and vigorous Anjou pear trees (French roots) in the Medford experiment station orchard were used. Tree X–40 had a trunk circumference of 73 cm and about 39,000 leaves. Tree MM–33 had a trunk circumference of 96 cm and about 70,000 leaves. The limb tips of tree MM–33, in a block planted approximately 26 feet on the square, nearly met those of adjacent trees; but between the limb tips of tree X–40, in a block planted approximately 30 feet on the square, and those of adjacent trees there was considerable space.

To isolate the soil mass around these two trees from the effect of roots from adjacent trees and from water seepage, trenches 20 inches in width were dug during March around four sides of a rectangle surrounding each tree. For Anjou X–40, in deep soil, the trench was excavated to the water table, a depth of 9 feet; for Anjou MM–33, in shallow soil, the trench was dug to a depth of 4 feet, or about 6 inches into the crumbling shale bedrock. After the inner sides of each trench were lined with single-ply tar paper fastened to boards extending 4 inches above the ground surface, the seams in the paper were tarred and the trench was filled in. The soil surface was left uncovered and all weeds were removed. The upward or downward movement of moisture from the soil mass was unobstructed by artificial barriers. With good natural drainage and practically no summer rainfall, no drainage of the soil mass, as provided in the experiments of Oskamp (*11*),[2] was necessary. For tree X–40 the isolated soil mass was 30 feet square; for tree MM–33 it was 27.0 feet long and 25.5 feet wide.

One sampling location was at the base of the tree. On each of one pair of opposite diagonals samples were taken at 2, 6, 10, 14, and 18 feet, respectively; on each of the other pair samples were taken at 4, 8, 12, and 16 feet, respectively.

To ascertain soil-moisture variations unaffected by active tree roots, similar walls were put around two cylindrical cores of undisturbed soil, 36 inches in diameter, one core being adjacent to each of the trees. Soil-moisture samples from these cores free of active roots were taken at the same dates as those from the root-containing area.

IRRIGATION PLOTS USED IN 1932

During 1932 additional information upon the relative soil-moisture decreases at different positions around moderate-sized Anjou trees was available in the soil-moisture data from four experimental irrigation plots. In these plots, each containing 0.88 acre and from 14 to 17 experimental trees, soil samples were taken in 1-foot increments to bedrock lying at depths of 4 to 6 feet, at definite locations 4, 7.5,

[2] Reference is made by number (italic) to Literature Cited, p. 987.

10, 11.5, and 13 feet from the trunks. In each plot, these positions were quadruplicated. Thus for each plot there were at each sampling from 16 to 24 separate determinations (four series, each consisting of one sample for each foot in depth) for each distance from the tree, and 20 separate determinations for each depth to 4 feet. The periods of soil-moisture decrease during the summer, selected for study of water extraction by the roots, were not the same for all plots.

SOIL-MOISTURE EXTRACTION AT DIFFERENT DEPTHS

After the spring cover crop had been disked under, one or more periods during the summer when soil-moisture decrease was rapid and unaffected by rainfall or irrigation were selected for each tree or group of trees. During these periods soil moisture did not decrease to the wilting point [3] at any depth. The average soil moisture content for each foot in depth, based on the average for all distances from the trunk, was calculated for the beginning and for the end of each selected period. The difference between these two averages gave the average soil-moisture decrease for any one depth during this period. Because the total amount of moisture extracted was not the same for all trees or plots, the amount of moisture extracted in each case was expressed as percentage of the sum of the moisture decreases for all depths. This percentage, considered the relative rate of moisture extraction for that depth, is then comparable with that for any other tree or plot.

For the Bartlett and Anjou trees studied in 1931, it was assumed that moisture loss by direct evaporation from the soil was too slight to be considered. The extent of such loss was measured in 1932 by the isolated cores of soil, containing no active roots. It was found that in the top foot of the core the moisture decreased for about 30 days, but in the lower depths moisture changes were negligible. The moisture decrease in the top foot of the core for tree X–40 was 0.07 percent per day, and for tree MM–33 it was 0.11 percent per day. Therefore, in calculating water extraction by the roots for trees X–40 and MM–33, this estimated loss was subtracted from the average soil-moisture decrease of the first foot only. Since evaporation loss from the four irrigation plots was not known, a correction of 0.092 percent per day (average of losses from trees MM–33 and X–40) was arbitrarily used for the first foot only in each plot.

The relative rates of extraction of moisture from each depth for Fitch orchard Bartlett, Klamath orchard Anjou tree X–40, tree MM–33, and the four irrigation plots are given in table 1.

The greater relative moisture extraction in the top foot of plot C (table 1) was perhaps partly due to the more frequent irrigation of this plot, which resulted in a continuously higher moisture content, probably with correspondingly greater surface evaporation loss, in the top foot of this plot than in the top foot of the other plots.

[3] In this statement, "soil moisture" refers to average moisture content of sample of soil taken with King tube.

TABLE 1.—*Relative rate of soil-moisture extraction at different depths and at different distances from the trunk for Fitch orchard Bartlett, Klamath orchard Anjou, tree X–40, tree MM–33, and the four irrigation plots*

AT DIFFERENT DEPTHS [1]

Depth or distance (feet)	Fitch orchard Bartlett	Klamath orchard Anjou	Tree X–40 [2]	Tree MM–33	Irrigation plot				Average [3] for trees in soil 4 feet deep
					A	B	C	D	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0–1	39	35	[4] 30	[4] 34	[4] 32	[4] 35	[4] 45	[4] 33	34
1–2	25	27	35	28	27	28	23	23	28
2–3	18	20	23	23	22	25	13	14	22
3–4	9	18	12	15	19	12	11	13	16
4–5	9	[5]	.....	[5]	[5]	[5]	8	12	.....
5–6	[5]	[5]	.....	[5]	[5]	[5]	[5]	5	.....

AT DIFFERENT DISTANCES FROM TRUNK [6]

0	50	55	75	120	.....	.....	.....	.....	75±10.7
2	80	80	100	135	.....	.....	.....	.....	99± 8.7
4	135	125	105	110	105	90	145	115	116± 4.3
6	90	95	105	100	.....	.....	.....	.....	98± 2.2
7.5 or 8	120	110	130	100	100	130	95	110	112± 3.2
10	85	80	85	90	95	95	95	100	91± 1.6
11.5 or 12	80	110	105	100	100	105	65	65	91± 4.4
13 or 14	80	75	75	100	100	80	100	110	90± 3.3
16	80	100	75	105	.....	.....	.....	.....	90±15.0
18	85	130	35	95	.....	.....	.....	.....	86±13.2

[1] Extraction at each depth is expressed as percentage of total extraction for all depths.
[2] Calculated for top 4 feet only, although soil was 9 feet deep.
[3] Based on Klamath orchard Anjou, tree MM–33, and irrigation plots A and B.
[4] Corrected for surface evaporation.
[5] Rock occurred at this depth.
[6] Extraction at each distance is expressed as percentage of average of extraction at 4, 8, 10, 12, and 14 feet for individual trees, and at 4, 7.5, 10, 11.5, and 13 feet for irrigation plots.

For the trees in soil just 4 feet deep, the first foot showed the greatest average extraction (34 percent); the top 2 feet, 62 percent; and the top 3 feet, 84 percent. The 3- to 4-foot zone showed only 16 percent. Although some roots penetrated the underlying shale bedrock, the moisture extracted by such roots was probably very small in proportion to that extracted in the top 4 feet.

SOIL-MOISTURE EXTRACTION AT DIFFERENT DISTANCES FROM TRUNK

In the determination of the soil-moisture extraction at each distance from the trunk, the average for all depths was used. Since the sampling locations for the individual trees were not all at the same distance from the trunk as were the locations for the irrigation plots, a special method of presentation has been used to make all the data comparable. The sampling locations for the individual trees at 8, 12, and 14 feet from the trunk are considered comparable to the locations at 7.5, 11, and 13 feet, respectively, for the irrigation plots. The relative rate of moisture extraction at any location for the individual trees has been expressed as percentage of the average extractions of locations 4, 8, 10, 12, and 14 feet from the trunk; whereas the relative rate of moisture extraction for any location in the irrigation plots has been expressed as percentage of the average extractions of locations 4, 7.5, 10, 11.5, and 13 feet from the trunk. The data, thus expressed, are given in table 1.

Both the individual trees and the irrigation plots show considerable variation in relative rate of moisture extraction at any given distance

from the tree. For the individual trees the small number of soil samples at each location might explain the variability, but for the irrigation plots the variation is more than might be expected for the average of 4 to 7 periods of extraction for four series of sampling holes. Such variation does not permit any meaning to be attached to tree or plot differences. However, the average relative rate of extraction for each approximate distance shows that the extractions at 4, 6, and 7.5 or 8 feet from the tree were slightly but not always significantly greater than at the base of the trunk or at distances greater than 8 feet from the trunk. At distances between 10 and 18 feet from the trunk the average rates of extraction were about the same.

ROOT DISTRIBUTION

ROOT COUNTS

When the trenches around trees X–40 and MM–33 were dug, the inner trench wall on two sides of each of the trees was divided into foot squares with chalk lines. When all roots along the wall were exposed by picking away the soil with a small ice pick, the number of roots below 1 mm in diameter in each square was recorded. The average number of such roots per square foot for each depth is given in table 2. Since there was no surface mulch, depths were measured from the soil surface.

TABLE 2.—*Average number of roots less than 1 mm in diameter for each soil depth for trees X–40 and MM–33*

[Data expressed as number of roots in 1 square foot on the side of a trench and as percentage of total]

Depth (feet)	Tree X–40 roots		Tree MM–33 roots		Depth (feet)	Tree X–40 roots		Tree MM–33 roots	
	Number	*Percent*	*Number*	*Percent*		*Number*	*Percent*	*Number*	*Percent*
0–1	9.6±0.58	11	14.8±0.74	28	4–5	2.0±0.10	2		
1–2	18.0± .81	21	16.6± .68	32	5–6	12.4±1.02	14		
2–3	19.9± .83	23	13.4± .55	26	6–7	7.3± .72	8		
3–4	12.4± .73	14	7.3± .47	14	7–9	6.3± .00	7		

For tree X–40 the greatest number of roots below 1 mm in diameter occurred in the second- and third-foot depths, with the next greatest number in the first-, fourth-, and sixth-foot depths; between 4 and 5 feet below the surface a sandy clay stratum containing deposits of lime had very few roots. Below 6 feet the silty clay-loam subsoil contained pockets of dark-colored clay, in which most of the roots at that depth were found.

The number of roots in each of the upper 4 feet, expressed as percentage of the total roots in the top 4 feet, was for the 0- to 1-foot depth, 16 percent; for the 1- to 2-foot depth, 30 percent; for the 2- to 3-foot depth, 33 percent; and for the 3- to 4-foot depth, 21 percent. This distribution of roots does not correspond to the relative rate of moisture extraction observed along diagonals extending out from the trunk (table 1). This lack of agreement may have been caused by vertical movement of soil moisture or by the difference in the root distributions at the soil-sampling locations from those in the trench profile. It is also possible that the root distribution nearer the trunk would differ somewhat from that obtained in a trench 15 feet from the trunk.

For tree MM–33, the first and second foot each contained about 30 percent of all the roots observed to a depth of 4 feet; the third foot contained 26 percent; and the fourth foot contained 14 percent. This distribution agrees fairly closely with the rate of moisture extraction at each depth for this tree (table 1), which showed 34, 28, 23, and 15 percent, respectively, in each foot between the surface and a depth of 4 feet.

ROOT WEIGHTS FOR TREE MM–33

In October 1932 the distribution of small visible roots around tree MM–33 was determined in order to learn how closely the concentration of such roots at different positions corresponded to the observed relative rate of moisture extraction. The distribution of visible roots was determined by a method somewhat similar to that reported by Rogers and Vyvyan (*16*) and by Furr and Magness (*6*). A trench 24 inches wide was dug along each of the four diagonals from the trunk to a distance of 14 feet from the tree. To a depth of 6 inches below the surface, the soil was removed separately from zones 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 14 feet, respectively, from the trunk. All the roots in each zone were separated from the lumps of sticky soil by hand, air-dried for approximately half an hour, separated into three groups according to size, and weighed to 5 mg. When the first 6 inches of soil in one trench had been removed, the next 6 inches was taken out in a similar manner; subsequently, the second 12 inches, the third 12 inches, and, finally, the lowest 12 inches. These operations were repeated for all four trenches. The weight of roots under 2 mm in diameter per cubic foot of soil (termed "root concentration") for each zone is given in table 3.

In view of the variability of root concentration for different locations, the trend of root distribution can be more clearly seen in the figures for average concentration for each depth and for each distance from the trunk.

The distribution of roots to a depth of 4 feet is shown in the next to the last column of table 3, which gives the average root concentration at each depth. It is evident that the greatest concentration of small roots occurred between 6 and 12 inches below the surface. This depth had nearly twice the average concentration of the 12- to 24-inch depth. The 24- to 36-inch depth and the 36- to 48-inch depth had each a lower root concentration than the layer just above. The 0- to 6-inch depth, which shows about one-quarter of the concentration found in the 6- to 12-inch depth, actually had almost no roots in the top 4 inches, as most of the roots were confined to a layer between 4 and 6 inches deep. The average concentration for the entire 0- to 12-inch depth, however, was about the same as that for the 12- to 24-inch depth.

The relative concentrations of roots for each foot in depth, expressed as percentages of the sum of the concentrations in the top 4 feet, given in the last column of table 3, were 35, 33, 21, and 11, respectively, for the first, second, third, and fourth foot in depth. These figures for root concentration are directly comparable with figures expressing the relative moisture extraction for these depths, which are (table 1) 34, 28, 23, and 15. Statistical comparison shows a correlation $+0.95 \pm 0.04$, indicating that for each depth average moisture extraction was directly proportional to the concentration of small visible roots.

TABLE 3.—*Concentration of visible pear roots less than 2 mm in diameter, for tree MM–33, in clay adobe soil 3½ feet deep to shale bedrock*

[Data given as grams of roots per cubic foot of soil]

Depth (inches)	Trench location	Roots in soil zone at indicated distance from trunk							Average root concentration	Relative root concentration [1]
		0–2 feet	2–4 feet	4–6 feet	6–8 feet	8–10 feet	10–12 feet	12–14 feet		
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
0–6	SW	1.6	4.1	4.4	4.0	2.7	3.0	1.4		
	SE	1.4	3.6	2.5	2.4	3.9	1.4	1.6		
	NW	1.5	.9	1.4	.8	2.3	2.1	2.7		
	NE	1.5	4.9	2.0	1.3	1.8	1.8	1.6		
	Average	1.5	3.4	2.6	2.1	2.7	2.1	1.8	2.3	
6–12	SW	10.7	10.0	8.8	8.5	7.8	6.0	4.7		
	SE	5.3	18.6	11.0	12.2	7.0	5.7	4.3		
	NW	5.2	9.6	10.9	10.1	7.9	10.6	5.1		
	NE	5.4	7.8	14.4	11.0	12.4	11.0	15.1		
	Average	6.7	11.5	11.3	10.5	8.8	8.3	7.3	9.2	
0–12	do	4.1	7.4	6.9	6.3	5.7	5.2	4.6	5.7	35
12–24	SW	6.0	5.0	5.7	5.0	5.2	6.5	7.6		
	SE	8.4	6.7	6.0	5.4	4.9	4.5	3.8		
	NW	4.4	8.4	8.3	5.7	2.9	2.8	5.1		
	NE		3.9	8.6	4.4	4.2	4.1	4.8		
	Average	6.3	6.0	7.2	5.1	4.3	4.5	5.3	5.5	33
24–36	SW		4.0	3.1	5.6	3.8	4.8	3.0		
	SE		3.8	4.3	5.2	3.9	4.1	3.8		
	NW	2.2	3.1	1.8	3.1	2.0	3.1	2.3		
	NE		3.8	2.6	3.4	2.8	4.2	3.3		
	Average	2.2	3.7	3.0	4.3	3.1	4.1	3.1	3.4	21
36–48	SW		.9	2.3	2.3	2.1	1.8	.9		
	SE		2.1	2.2	2.1	2.7	2.0	2.1		
	NW	1.5	3.7	2.1	.5	.5	.5	1.1		
	NE		1.7	1.6	2.5	1.1	1.5	2.6		
	Average	1.5	2.1	2.1	1.9	1.6	1.5	1.7	1.8	11
0–48	do	3.5	4.8	4.8	4.4	3.7	3.8	3.7	4.1	
	Relative root concentration [2]	*Percent* 83	*Percent* 114	*Percent* 114	*Percent* 105	*Percent* 88	*Percent* 90	*Percent* 88		

[1] Percentage of total roots in top 4 feet.
[2] Percentage of average concentration between 4 and 12 feet from trunk.

The lateral distribution of roots from the trunk is indicated by the average root concentration for all depths (based on averages for entire 12-inch depth increments) for each distance from the trunk. These averages show a slightly lower root concentration between 0 and 2 feet from the trunk and between 8 and 14 feet from the trunk than between 2 and 8 feet from the trunk. However, these differences are not great, indicating that roots penetrate soil zones in the middle of tree rows nearly as well as those zones nearer the trunk.

Relative root concentrations at each distance from the trunk are shown in the last line of table 3, with concentration expressed as percentage of the average of the concentrations between 4 and 12 feet. Thus the relative root concentrations in the 0- to 2-foot, 2 to 4-foot, 4- to 6-foot, 6- to 8-foot, 8- to 10-foot, 10- to 12-foot, and 12- to 14-foot zones are 83, 114, 114, 105, 88, 90, and 88, respectively. The relative rates of moisture extraction (table 1) at points 0, 2, 4, 6,

8, 10, 12, and 14 feet from the trunk were 120, 135, 110, 100, 100, 90, 100, and 100, respectively. Since root-distribution values are for soil zones 2 feet long, moisture extractions for zones, instead of for points, were estimated by averaging the relative moisture extraction at both ends of each zone. By this means relative moisture extractions for zones 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 14 feet from the trunk became 127.5, 122.5, 105, 100, 95, 95, and 100, respectively. These figures for relative root concentration and relative moisture extraction at each distance from the trunk show a zero correlation. This lack of correlation results principally from the lack of agreement between root concentration and moisture extraction in the 0- to 2-foot and 2- to 4-foot zones. Since moisture extraction at 0 feet from the trunk was based upon only one determination for each of the four depths in one sampling hole, the value 127.5 for relative moisture extraction for the 0- to 2-foot zone may be much less reliable than the other relative moisture-extraction values. With the 0- to 2-foot zone omitted, correlation between root concentration and moisture extraction becomes $+0.69 \pm 0.16$.

The root concentrations in table 3 show an unevenness in root distribution. Thus in the southwest trench in the upper 6 inches between 2 and 4 feet from the trunk, there were 4.1 g of roots per cubic foot, whereas in a similar soil zone in the northwest trench there were only 0.9 g of roots. Table 3 shows many such variations in distribution. Where the higher concentrations were not near large roots, it was not possible in the heavy clay soil to determine whether the root growth occurred at the end of vigorous lateral roots or whether it was stimulated as a result of the cutting of roots by cultivation implements, as the work of Kinman (7) suggests. However, regardless of the cause, the uneven root distribution observed around such a large tree in soil only 3½ feet deep shows one very important point, namely, that penetration of the soil mass by the small roots (less than 2 mm in diameter) was not uniform.

Furthermore, it was observed that some small soil volumes contained no visible roots. It is not likely that roots too small to be observed had penetrated soil volumes in which no visible roots were found. Thus it would seem that in this heavy clay soil, root distribution is not only uneven but apparently entirely lacking in small volumes in the soil mass.

ROOT WEIGHTS FOR OTHER TREES

Additional data upon root distribution were provided by trenches dug on two sides of trees E–6 and L–12 at the Medford experiment station and on one side of trees E–4 and B–7 in the Klamath orchard. Each of these four trees had a much smaller spread of limbs than tree MM–33. The trenches were 18 inches wide and 36 inches deep. The roots were removed and weighed in a manner similar to that used for tree MM–33. The data for these four trees, together with comparable data for tree MM–33, are summarized in tables 4 and 5.

Table 4 gives the relative root concentration at each depth, thus expressing root concentration as percentage of the total of the root concentrations between the surface and a depth of 3 feet. Individual trees show rather large differences in relative root distribution, but the data for each tree are too limited to justify explanation on the

basis of tree size or soil management. The average for trees E–6, L–12, E–4, and B–7 and the average for tree MM–33 agree extremely well. The averages for all 10 trenches (5 trees) were determined and the probable errors of the averages were calculated on the assumption that the data for all trenches were comparable.

TABLE 4.—*Relative concentration of visible pear roots, under 2 mm in diameter, in the top 3 feet in heavy clay soil, from 3½ to 6 feet deep to bedrock*

[Relative concentration of roots is expressed as percentage of the sum of the concentrations in the top 3 feet]

Trench designation	Roots in soil zone at indicated distance below ground		
	0–1 feet	1–2 feet	2–3 feet
	Percent	*Percent*	*Percent*
Klamath B–7	48	34	18
Klamath E–4	23	40	37
E–6, N	35	36	29
E–6, S	35	36	29
L–12, N	53	30	17
L–12, S	45	37	18
Average	40	35	25
MM–33, SW	36	36	28
MM–33, SE	38	34	28
MM–33, NW	44	37	19
MM–33, NE	45	34	21
Average	41	35	24
Average of 10 trenches	40.2	35.4	24.4
Probable error of average	±1.8	±0.6	±1.4

TABLE 5.—*Relative concentration of visible pear roots, under 2 mm in diameter, in the top 3 feet at different distances from the trunk, for heavy clay soil from 3½ to 6 feet deep*

[Relative concentration of roots is expressed as percentage of the average concentration between 4 and 12 feet from the trunk]

Tree designation	Roots in soil zone at indicated distance from trunk					
	0–2 feet	2–4 feet	4–6 feet	6–8 feet	8–10 feet	10–12 feet
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Klamath B–7	75	103	130	110	114	46
Klamath E–4		129	118	103	100	79
E–6, N	53	82	99	173	71	57
E–6, S		107	97	113	116	74
L–12, N	106	147	129	108	98	65
L–12, S		108	100	97	108	95
Average	78	113	112	117	101	69
MM–33, SW		103	99	108	92	101
MM–33, SE		140	111	117	93	79
MM–33, NW	76	127	123	108	76	93
MM–33, NE		91	125	90	91	94
Average	76	115	115	106	88	92
Average of 10 trenches	[1] 78	114	113	113	96	78
Probable error of average	±7.3	±4.5	±2.9	±4.8	±3.2	±3.9

[1] Average of four trees.

To make the values for relative root concentration directly comparable with values for average relative soil-moisture extraction for each of 4 top feet (table 1), these relative concentrations for the top 3 feet were recalculated on the basis of the top 4 feet. The percentage concentration in the 3- to 4-foot depth was assumed to be 11, which was the observed concentration in the 3- to 4-foot zone for tree MM–33. Thus the relative concentration of roots in the 0- to 1-, 1- to 2-, 2- to 3-, and 3- to 4-foot depths would be 36, 31, 22, and 11, respectively. The average relative moisture extraction for similar zones (table 1) was 34, 28, 22, and 16, respectively. This average distribution of root concentration, based on 10 trenches around 5 trees, compared with the average relative moisture extraction observed at similar depths around comparable trees, shows the close correlation of $+0.98 \pm 0.01$.

Table 5 gives the relative root concentration, based on all depths, for each distance from the trunk. The results are expressed as percentages of the sum of the root concentrations between 4 and 12 feet. The averages for trees E–6, L–12, E–4, and B–7 agree fairly closely with the averages for the four trenches around tree MM–33. To further summarize the results, the average for all trenches is presented, together with the probable error of the average calculated on the assumption that all trenches were comparable. These averages show that the zones 2 to 4, 4 to 6, and 6 to 8 feet from the trunk had about equal root concentrations, the zone 0 to 2 feet from the trunk and the zones 8 to 10 and 10 to 12 feet from the trunk having lower concentrations.

From the average relative moisture extractions at points at different distances from the trunk (table 1), the average relative extractions for zones 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, and 10 to 12 feet from the trunk were calculated and found to be 87, 107.5, 107, 105, 101.5, and 91, respectively. A comparison of these relative moisture-extraction values with the relative root concentrations for similar zones (78, 114, 113, 113, 96, and 78, respectively) shows a correlation of $+0.96 \pm 0.02$.

DISCUSSION

The decrease in soil moisture between field capacity and permanent wilting percentage in heavy clay soil surrounding the roots of large pear trees represents either surface evaporation or extraction by pear roots. It was found from soil cores without active roots that surface evaporation affected moisture only in the top foot. When the soil moisture decreases in the top foot had been corrected for surface-evaporation loss, the soil moisture decreases were considered measures of the amount of moisture extracted by the roots.

Although it is probable that soil-moisture movement from the soil into the roots largely occurred through the root hairs and rootlets too small to be observed and weighed by the method used, it was assumed that the water-absorbing area of such root hairs and rootlets was approximately measured by the weight of the small visible roots picked out of the heavy clay soil.

It was found that the relative rates of moisture extraction by the roots at different soil depths and at different distances from the trunk were, in general, directly proportional to the relative concentrations of small visible pear roots in corresponding locations. In

the case of tree MM–33 no linear correlation was found between root concentration and rate of moisture extraction. However, the rate of extraction from the 0- to 2-foot zone is based in part on a single hole at the base of the tree. When the data for the 0- to 2-foot zone are omitted the coefficient of correlation is +0.69.

The correlation for tree MM–33 was +0.95 for all depths. However, when the averages of relative moisture extraction for the 4 individual trees and the 4 irrigation plots were compared with averages of relative root concentration for 10 trenches around an aggregate of 5 trees, the coefficient of correlation in relation to depth was +0.98 and the coefficient of correlation in relation to distance from the trunk was +0.96. Thus, when the averages were based on a sufficient number of determinations to reduce the influence of wide individual variations, the average relative moisture extraction was closely correlated with the average relative concentration of small visible roots. In other words, the rate of extraction of available moisture from the soil seems dependent upon the concentration of the small roots. Somewhat similar results were obtained by Conrad and Veihmeyer (*5*), who, working with a row of sorghum plants in relatively light soil, found a positive correlation between the extent of soil dryness and the amount of roots.

Beckett, Blaney, and Taylor (*4*), studying citrus and avocado in sandy loam 3 to 5 feet deep, report "apparent root development", as determined from the rate of water extraction, greatest in the first foot and materially decreasing with depth.

The positive correlation between the amount of soil moisture extracted from a specific soil mass and the pear root concentration therein also indicates that the weight of small visible roots per unit volume of soil provides a measure of root area active in water intake. Thus in regions where the transpiration needs of the trees are great, as in the Rogue River Valley, the water-supplying capacity of the roots should be judged by the distribution of zones of high root concentrations rather than by the presence of a few roots.

The fact that after moisture extraction by the roots different soil zones contained different amounts of moisture shows that capillary movement of moisture through the soil was not sufficient to equalize differences in rate of moisture removal by the roots. This is in agreement with the conclusions of McLaughlin (*9*), Veihmeyer (*17*), and others that capillary movement of moisture from soil relatively high in moisture to soil that is being dried out by root extraction is not sufficiently great to render all moisture in the soil mass equally available to the tree. The import for practical pear irrigation is obvious. In the determination of the time or amount of irrigation necessary for the trees, observations of soil moisture should be based on soil samples taken from zones of relatively great root concentration.

From the weights of the visible pear roots (less than 2 mm in diameter) per cubic foot of soil, it was found that the highest concentration of roots occurred between 4 and 12 inches below the surface. On the basis of four trenches around one large tree, it was found that of all the roots to a depth of 4 feet (bedrock 3½ to 4 feet deep) 35 percent were in the top foot, 33 percent in the second foot, 21 percent in the third, and 11 percent in the fourth. Thus the top 2 feet contained 68 percent of the total roots in the upper 4 feet, and the upper 3 feet

contained 89 percent. These results agree with those obtained in extensive apple root examinations by Oskamp and Batjer (*13*), who found a greater concentration in the first 8 inches than in the second 8 inches and 60 percent or more of the roots in the top 16 inches; with those of Oskamp (*12*), who found cherry root concentration greatest in the top foot; and with those of Beckenbaugh and Gourley (*3*), who found that apple root concentration decreased from the surface down. Rogers and Vyvyan (*16*) found the same relation between depth and apple root concentration, except that the root concentration was greater between 17 and 44 cm below the surface than between 0 and 17 cm below the surface. More casual observations by Allen (*1*), Ballantyne (*2*), Lunt (*8*), Oskamp (*11*), and Partridge and Veatch (*14*) showed the greater portion of the root mass to be in the upper 1 or 2 feet. Such a relatively high proportion of the roots near the surface should be kept in mind when considering subsoiling, the depth to apply fertilizers, and the soil mass in which to maintain available moisture by irrigation.

As to the lateral distribution of small pear roots in 4 trenches around 1 large tree and in 6 trenches around a total of 4 other trees, the concentrations between 2 and 8 feet from the trunk were found to be slightly greater than those between 0 and 2 feet or between 8 and 14 feet from the trunk. However, the concentrations between 8 and 12 feet from the trunk, although in most cases significantly less than those between 2 and 8 feet, were much higher than those for apple roots in lighter soils. Furr and Magness (*6*), Morris (*10*), Rogers and Vyvyan (*16*), Rogers (*15*), and Beckenbaugh and Gourley (*3*) found that root concentration decreased appreciably with an increase in lateral distance from the trunk. Although Rogers and Vyvyan were studying rather small trees, the tree studied by Magness and Furr and the trees in orchard A examined by Beckenbaugh and Gourley were fully as large as the pear trees considered in this report. The large pear root concentrations at distances of 8 to 14 feet from the trunk in the heavy soil studied indicate that fertilizer and irrigation water for trees in this type of soil should, for availability to the maximum root area, be applied to the entire area between the tree rows.

SUMMARY

The amount of soil moisture extraction is positively correlated with the concentration of the small visible roots, which seems to be a measure of concentration of rootlets and root hairs too minute to be observed by the method reported.

Capillary movement of soil moisture is not sufficiently rapid to equalize differences in the amount of moisture extracted from different soil zones.

Root concentration is not uniform throughout the soil mass, and many small volumes of soil contained no visible roots.

Of the total moisture extracted from the upper 4 feet of soil, about 34 percent was extracted from the top foot, about 62 percent from the top 2 feet, and about 84 percent from the top 3 feet.

Of the total root concentration in the upper 4 feet of soil, about 35 percent was in the top foot, about 68 percent in the top 2 feet, and about 89 percent in the top 3 feet.

The most rapid moisture extraction by the roots occurred between 2 and 8 feet from the trunk, and 5 to 15 percent less rapid extraction occurred between 8 and 14 feet from the trunk.

The greatest root concentrations were found between 2 and 8 feet from the trunk, lower concentrations between 8 and 12 feet from the trunk.

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THE MEXICAN BEAN BEETLE IN MEXICO [1]

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INTRODUCTION

The Mexican bean beetle (*Epilachna corrupta* Muls.) is a destructive pest of beans in Mexico. Although this insect is apparently indigenous to the country and has a wide range of distribution, little is known

FIGURE 1.—Distribution of the Mexican bean beetle in Mexico.

concerning its biology. A study of the Mexican bean beetle was therefore undertaken at Mexico City in 1930.

[1] Received for publication Feb. 6, 1935; issued July 1935. This study was made at the suggestion of N. F. Howard, in charge of pea and bean insects, Division of Truck Crop and Garden Insect Investigations, Bureau of Entomology and Plant Quarantine. It was carried on in cooperation with the Mexican Department of Agriculture.

[2] Now with the Division of Fruit Insects.

[3] Thanks are due Alfonso Dampf, of the Mexican Department of Agriculture, for assistance in procuring altitude records and to Leopoldo de la Barreda for making unpublished distribution records available.

DISTRIBUTION

Although many legumes are grown in Mexico, beans seem to be the preferred food plant of the bean beetle. In the States of Guanajuato, Zacatecas, Jalisco, Michoácan, Aguascalientes, Queretaro, Durango, Puebla, Veracruz, and San Luis Potosí, where commercial acreages of beans are grown, the bean beetle causes destruction to the crop periodically. Dampf (*9*) [4] says that, in all parts of the Republic where beans are cultivated, agriculturists know the bean beetle as the most terrible pest of this legume, occasionally causing complete loss of the crop. This is contrary to Sweetman's statement (*11, p. 226*) that "The insect is not a serious pest over much of Guatemala and Mexico * * *." Beans are grown from sea level to an elevation of approximately 10,000 feet, but the most extensive cultivation is found within these States, principally on the broad, central plateau having an elevation of 1,500 to 2,000 feet in the north and 7,000 to 8,000 feet or more in the Valley of Mexico or the vicinity of Mexico City (fig. 1). The 20° C. isotherm roughly depicts the limits of this plateau.

TABLE 1.—*Localities in Mexico where the Mexican bean beetle has been recorded*

Locality	Altitude	Reference [1]
	Feet	
Federal District:		
Contreras	Over 7,500	X.
Mexico City	7,349	(*3*), X.
San Jacinto	7,349	(*7*), X.
Tacuba	7,349	R.
Xochimilco	7,349	X.
Mexico (State of):		
Acozac		(*3, 10*).
Amecameca	8,189	X.
Apaseo [Apaxco]		(*8, Bol. 2, p. 558*).
Chalco	7,450	(*10*), X.
Chicoloapam		(*10*).
Hacienda del Prieto [Naucalpan]		R.
Lechería	Over 7,000	R.
Naucalpan		R (*Epilachna*).
San Andrés Atenco [Tlalnepantla]	Over 7,000	(*8, Bol. 3, p. 501*).
Temamatla		(*10*).
Temascalcingo		R.
Toluca	8,845	X.
Tenango	Over 8,000	(*10, 1*).
Texcoco	7,349	(*10*), X.
Zacualtipan, Estado General González		R.
Morelos:		
Cuautla	4,235	X.
Cuernavaca	5,059	X (*1*).
Puente de Ixtla	3,200	X.
Puebla:		
Acatzingo		R.
Actopan		(*10*).
Amozoc		(*8, Bol. 2, p. 559*).
Atlixco	6,171	(*1*), X.
Chalchicomula	8,333	(*8, Bol. 2, p. 558*).
Chiautzingo	7,516	(*7*).
Cholula	7,054	(*1*), X.
Cuetzalan del Progreso [Zacapoaxtla]		R.
Huejonapa [Huejonapan]		R.
Huixcotla-Tecamachalco	6,742	R, (*10, 3*).
Malpaís		(*8, Bol. 2, p. 558*).
Matamoros Izúcar	4,350	(*1*).
Nopalucan, Hacienda de "La Florista"		(*8, Bol. 2, p. 332; 10*).
Puebla	7,093	(*1, 3*), X.
Quecholac		R.
Rancho de San Cayetano, Tecamachalco		(*3, 10*).
San Cristóbal, Texmelucan		R.
San Hipolito		(*8, Bol. 3, p. 245*).

[1] Numbers refer to literature cited from which records were obtained, the volume and page number being given where confusion might arise; X indicates observations made by the authors; and R indicates records from the files of the Mexican Department of Agriculture.

[4] Reference is made by number (italic) to Literature Cited, p. 1001.

TABLE 1.—*Localities in Mexico where the Mexican bean beetle has been recorded*—Continued

Locality	Altitude	Reference
	Feet	
Puebla—Continued.		
San Jerónimo Xayacatlán		R.
San Marcos		(*10, 3*).
San Martín Texmelucan		R.
Santa Ana Coapan [near Puebla]		(*8, Bol. 3, p. 245*).
Tecali		(*10, 3*), R.
Tepatlaxco		R.
Tepeaca	7,405	(*8, Bol. 2, p. 332*).
Texmelucan		(*8, Bol. 3, p. 501*).
Tlaixpan		R.
Tlalancaleca		(*8, Bol. 2, p. 848*).
Veracruz:		
Córdoba	2,756	(*1*).
Jalapa	4,681	(*1*), X.
La Charca Atoyac	Nearly 1,500	R.
Orizaba	4,212	(*1*).
San Andrés Tuxtla	1,184	R.
Veracruz	6	(*7, p. 248*)[1].
Guerrero:		
Chilpancingo	4,461	(*1*).
Omilteme [Rancho de, Chilpancingo?]		(*1*).
San Marcos		R.
Xucumanatlan		(*1*).
Oaxaca:		
Guelavía, Distrito Tlacolula	Over 5,000	R.
Juquila		(*1*).
Matatlán, Distrito Tlacolula	Over 5,000	R.
Oaxaca City	5,068	(*1*), X.
Quiavini, Distrito Tlacolula	Over 5,000	R.
San Marcos Tlapasola, Distrito Tlacolula	Over 5,000	R.
Hidalgo:		
Bomintzá		(*7*).
Mixquiahuala de Juárez		R.
Rancho del Refugio Atotonilco de Tula		R.
Tepeji del Río		(*7*).
Tula	6,778	(*8, Bol. 3, p. 501*).
Queretaro:		
Cadereyta de Montes	6,814	R.
Querétaro	6,079	(*3*).
San Juan del Río	6,489	(*7*).
Michoacan:		
Aporo		R.
Maravatío	6,824	(*10, 3*).
San Andres Coru		R.
San Miguel el Alto		R.
Nayarit:		
Chapalilla		(*8, Bol. 2, p. 559*).
Tepic	3,067	(*8, Bol. 3, p. 501*).
Colima:		
Colima City	1,656	R (*Epilachna*).
Guanajuato:		
Allende [San Miguel Allende]	6,135	R (*Epilachna*).
Celaya	5,931	R.
Dolores Hidalgo	6,519	R.
Guanajuato	6,725	(*1; 8, Bol. 2, p. 559*).
Hacienda de Carranci [Cortazar]		(*3, 10*).
Hacienda de Noria de Charcas, San José de Iturbide		(*10*).
Salamanca	5,646	(*10*).
San Diego de la Unión		(*8, Bol. 2, p. 848*).
San José de Iturbide		(*10*).
San Luis de la Paz		R.
Taranandacuau [Tarandacuao?]		R.
Jalisco:		
Guadalajara	4,987	X.
Hacienda de Las Pilas		(*10*).
Huejuquilla el Alto		R.
La Grulla Autlán		R.
Mezquital		R.
Orendaín [near Refugio]		(*8, Bol. 2, p. 558*).
San Martín Hidalgo		R.[2]
Villa de Guadalupe		(*10, 3*).
Aguascalientes:		
Rincón de Romos		(*10*).
San Luis Potosí:		
Estación Micos		(*8, Bol. 2, p. 148*).
Hacienda de Bledos [Bledos]		(*1*).
Alvarez Mountains		(*1*).
San Luis Potosí	6,158	R (*8, Bol. 3, p. 501*).

[1]Doubtful record. There is no certainty that *Epilachna corrupta* is the species to which reference is made.

TABLE 1.—*Localities in Mexico where the Mexican bean beetle has been recorded*—Continued

Locality	Altitude	Reference
	Feet	
Tamaulipas:		
Ciudad Ocampo	1,142	(*10, 5*).
Tampico	3	(*11*).
Durango:		
Barrazas	Nearly 6,696	(*8, Bol. 3, p. 501*).
Canacatlan [Canatlán]		R.
Durango	6,197	(*7*), R.
Hacienda de El Salto [Pueblo Nuevo]		R.
Nombre de Dios	Nearly 6,500	(*10*).
Pánuco de Coronado		(*10*).
San Bartolo		(*10*).
San Bernardo el Oro		(*10*).
San Gabriel		(*10*).
San Juan de Guadalupe	Nearly 5,000	(*10*).
San Juan del Río		(*10*).
San Pedro Otáez		(*8, Bol. 3, p. 501*).
Santiago Papasquiaro	6,696	R.
Suchil		R.
Tepehuanes		(*8, Bol. 3, p. 501*).
Villa General Vicente Guerrero		(*8, Bol. 3, p. 501*).
Coahuila:		
Monclova	1,758	(*1*).
Saltillo	5,246	(*1*).
Section around Torreón	3,641	(*8, Bol. 2, p. 558*).
Chihuahua:		
Batopilas [Batopilillas]	5,105	R.
Casas Grandes	Nearly 4,777	R.
Chihuahua	1,564	(*1; 8, Bol. 2, p. 558*).
Chínipas	Over 5,000	R.
Ciudad Juárez	3,753	R.
Hacienda de San Simón Guadalupe y Calvo		R.
Hidalgo del Parral	5,449	R.
Ojinaga	2,759	R.
Yoquivo [Real Yoquivo]		R.
Sonora:		
Atil		(*7*).
Cucurpe		(*10*).
Estación Esqueda		R.
Magdalena	2,595	(*10*).
Tlaxcala:		
Calpulálpan		(*1*).
Contla		(*7, p. 128*) [1].
San Bartolo Tepujahualco [Tepeyahualco]		R.
San Pablo del Monte		R.
Zacatecas:		
Canutillo		(*8, Bol. 3, p. 501*).
Sombrerete		(*8, Bol. 3, p. 501*).
Unknown localities:		
Hacienda del Rincón		(*3*).
Presidio		(*1*).

[1] Doubtful record. There is no certainty that *Epilachna corrupta* is the species to which reference is made.

Locality records from the Biologia Centrali-Americana (*1*), unpublished records and publications of the Mexican Department of Agriculture (*7*, *8*, *9*, *10*), and field observations of the writers have shown the bean beetle to be present in 22 States and the Federal District (table 1). These records are not complete, but they show the wide distribution of the insect in the better known parts of Mexico. Unfortunately it has been impossible to procure altitude records for all the places in which the Mexican bean beetle has been found. However, it may be noted from the altitudes given that most of these places are from 5,000 to 7,000 or more feet above sea level. The extremes of elevation are 3 feet at Tampico, Tamaulipas, and 8,845 feet at Toluca, Mexico. Most of the records came from the States of Mexico, Puebla, Durango, Guanajuato, and Chihuahua, all of which are situated on the central plateau. Whether this is due to the fact that more beans are grown in these States and hence more

people report damage, or whether the damage actually is greater there than elsewhere, is not known.

Uvarov (*12, p. 130*) states: "This beetle is a serious pest in the parts of Mexico which have a hot and very damp climate * * *." However, with the exception of infestations reported from the vicinity of Tampico, Tamaulipas,[5] Colima, Colima, and Veracruz, and possibly San Andres Tuxtla and La Charca Atoyac, Veracruz, the bean beetle does not appear to be present in the hot, damp coastal regions. Were it present or doing considerable damage, we should expect to find more records from the State of Veracruz, where commercial acreages of beans are grown.

SEASONAL ACTIVITY

At various times during the growing season of 1930 a few localities outside of the Federal District were visited in order that the situation as to bean beetle infestation might be ascertained. The results of these visits are summarized in table 2.

TABLE 2.—*Mexican bean beetle infestation in various localities in Mexico on different dates in 1930*

Date	Place	Elevation	Bean beetle situation
		Feet	
Apr. 28	Tampico, Tamaulipas [1]	3	No beans growing.
June 5	Veracruz, Veracruz [1]	6	Beans not infested.
6	Jalapa, Veracruz [1]	4,681	One adult beetle taken.
11	Puente de Ixtla, Morelos	3,200	Beetles found.
15	Monterrey, Neuvo Leon	1,771	No beans found.
17	San Luis Potosi, San Luis Potosi [1]	6,158	Do.
July 1	Cordoba, Veracruz [1]	2,756	Do.
4	Cuernavaca, Morelos [1]	5,059	All stages; severe damage.
9	Chilpancingo, Guerrero [1]	4,461	No beans found.
10	Acapulco, Guerrero	3	Do.
16	Cholulu, Puebla [1]	7,054	All stages; severe damage.
	Atlixco, Puebla [1]	6,171	Do.
Aug. 8	Guadalajara, Jalisco	4,987	Few larvae taken.
22	Oaxaca City, Oaxaca [1]	5,068	Young beetles and few larvae found.

[1] The bean beetle has been reported from this place (table 1).

In sections where there is a pronounced dry season, the bean beetle is present in the fields only during the wet summer months. There is considerable variation in annual precipitation within the area of known bean beetle distribution. In the vicinity of Jalapa and Cordoba it ranges from 60 to 85 inches and is distributed throughout the spring, summer, and fall. Around Torreon, in northern Mexico, the rainfall is only about 10 inches, and this occurs almost entirely during the summer months. In general, the period of summer rainfall is shorter and the precipitation less as the elevation and distance inland, northward, and westward from the Jalapa-Cordoba district increase.

A climograph (hythergraph) for Mexico City published by Graf (*2*) shows that the monthly temperature rises rapidly from January to May and declines during the 4 rainy months that follow. If temperature is the deciding factor in breaking the dormancy of the bean beetle, we should expect to find the beetle on beans grown under

[5] ROBLEDO, F. G. OUTSTANDING ENTOMOLOGICAL FEATURES FOR MEXICO FROM JANUARY TO JUNE 1931. U. S. Dept. Agr., Bur. Ent. Insect Pest Survey Bull. 11:412-416. 1931. [Mimeographed.]

irrigation during April and May in Cuernavaca, since similar temperature changes occur there. The fact that the bean beetle does not appear in Cuernavaca and Mexico City until the summer rainy season indicates that moisture and increasing temperature furnish the stimulus required to bring the beetles from localities where they have passed the dormant period. The small summer crop of beans is grown in Cuernavaca without irrigation, and all the plants are defoliated by the beetle.

In 1929 some beans that had been planted in a protected spot at the Mexico City laboratory escaped the October frosts. Beetles and larvae migrated to these plants from other beans that had been killed by defoliation and frost. A sprinkler was used for several hours each day in order to maintain a high humidity. Beetles remained on these plants, and all stages of the insect were found there as late as February 3, when the observations were discontinued. This was 4 months after their normal occurrence in the field. Later work by the senior author in Columbus, Ohio, has shown that the bean beetle can easily be reared through the winter months under greenhouse conditions.

CLIMATE OF MEXICO CITY

According to Marcovitch and Stanley (*5, p. 676*):

> During July and August, the months most favorable for bean growing, maximum temperatures of but 70° F. are reached in Mexico City (Hernandez, 1923). * * * May, the hottest month in the year, has a maximum temperature of but 75° F. These comparatively low temperatures and the generous rainfall of 16 inches for the summer months, are the climatic conditions the bean beetle has been exposed to for numberless generations and undoubtedly present the optimum requirements for breeding.

In another place (*4*) Marcovitch gives the mean summer temperature as 60° to 65° F. and the rainfall as 4 inches.

An atlas published by the Mexican Department of Agriculture (*6*) summarizes the temperature records [6] taken at the Tacubaya (Federal District) station (about 4 miles from San Jacinto and at a slightly higher elevation) for the period 1921–25. The mean daily temperatures were as follows: March, 17.8° C.; April, 17.1° C.; May, 17.6° C.; June, 16.4° C.; July, 15.4° C.; August, 15.7° C. The maximum temperatures are not given, but the mean temperatures indicate that the maximum temperatures were higher than those given by Marcovitch and Stanley (*5*). The same atlas (*6*) shows that the total precipitation at the Tacubaya station, as averaged for 1921–25, was 411.4 mm for June, July, and August, and 582.2 mm for the 4 months from June to September, inclusive. The records for San Jacinto (*6*), within a quarter of a mile from the place in which the present studies were made, for the same periods were 356.5 and 492.3 mm, respectively.

Thus there are several conflicting records for the precipitation and temperature in the vicinity of Mexico City. This may be due to the fact that there are several meteorological stations in the Federal District, which might be designated as Mexico City, and the records of these stations vary considerably although they are less than 4 or 5 miles apart. It is, however, incorrect to base conclusions concerning the natural habitat of *Epilachna corrupta* on such information.

[6] Obtained by taking the mean of the maximum and minimum daily temperatures and averaging them to get the monthly mean temperature.

In 1930 the writers placed a sheltered hygrothermograph 12 inches above ground in the middle of a bean field where life-history studies were being made. A summary of the temperature records taken on this instrument from July 1 to September 30, as given in table 3, indicates that the mean maximum temperature for the summer months was above 25° C. The weather that year was normal. The maximum daily temperature was recorded at about 2 p. m. and was maintained for but a short time. After the daily rain the temperature dropped rapidly until about 4 a. m.

TABLE 3.—*Summary of daily temperature records at Mexico City (San Jacinto), July 1 to September 30, 1930*

Record	July	August	September	For 3 months
	°C.	°C.	°C.	°C.
Maximum:				
High	30.0	30.0	34.5	
Low	23.0	20.0	25.0	
Mean	25.7	27.1	29.9	27.8
Minimum:				
High	14.0	13.0	13.0	
Low	8.0	7.0	2.5	
Mean	11.3	9.7	9.1	10.1
Daily mean:				
High [1]	19.2	19.4	20.1	
Low [1]	14.6	14.9	14.9	
Monthly mean	16.7	17.2	17.7	17.2

[1] Summary of readings at 2-hour intervals.

The beginning of the rainy season of 1930, like that of 1929, preceded the emergence of the beetles from dormancy by approximately 2 weeks. During this season there was, as a rule, some precipitation every day, usually late in the afternoon or in the evening. The daily average relative humidity was close to 70 percent and showed little variation from early in June until the middle of September, when the rains became less frequent.

Other environmental factors, such as light and barometric pressure, may have more than a minor influence, directly or indirectly, on the bean beetle. At the altitude and latitude of Mexico City the sunlight is intense and contains more ultraviolet than at higher altitudes. At this altitude (7,349 feet) the mean barometric pressure is only 23.43 inches.

The writers consider that the field data obtained on the temperature and the number of generations a year do not indicate optimum climatic conditions for breeding the bean beetle. The low daily mean temperatures preclude the development of more than a single generation of *Epilachna corrupta* in the vicinity of Mexico City. Marcovitch and Stanley, after saying (*5, p. 676*) that climatic conditions in Mexico present "optimum requirements for breeding", also say (*5, p. 769*) that "At 25° C. the greatest percentage reached maturity, so that this temperature may be considered as the optimum." The work reported in this paper will show that 25° is more likely to be the optimum temperature than the temperatures that prevail in Mexico City.

EXTENT OF BEAN BEETLE INFESTATIONS IN VICINITY OF MEXICO CITY

The severest infestations in the vicinity of Mexico City in 1929 and 1930 were found in a large, dry lake bed between the villages of Mixquit and Chalco, 33 miles southeast of Mexico City. The soil here is alkaline and of a fine, silty texture. Mountains are not far distant. Each year this lake bed is planted to corn and interplanted with beans. There was considerable variation in the degree of infestation in this lake bed; in some places the plants were completely defoliated while in others there was little or no injury. It is estimated that less than half the plants showed any great injury. This variation did not seem to be due to any difference in the varieties of beans

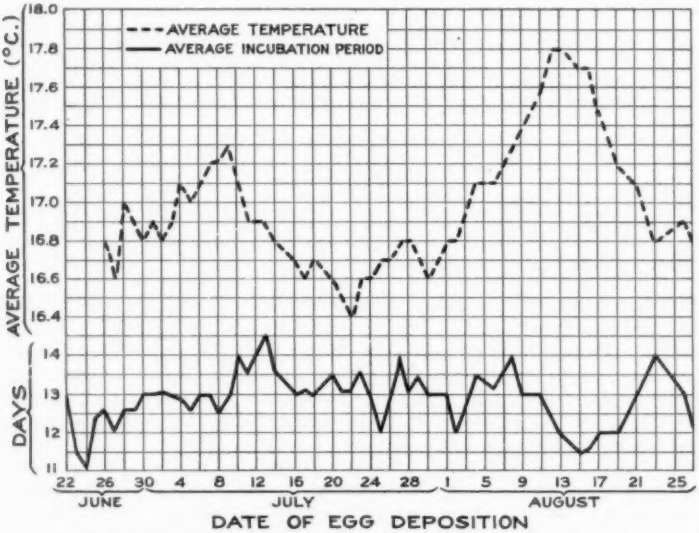

FIGURE 2.—Relation between the length of the incubation period of egg masses of the Mexican bean beetle and the temperature. The lower curve shows the average number of days required for the incubation of varying numbers of egg masses deposited each day during the season; the upper curve shows the average temperature during each of these incubation periods.

grown or to differences in soil or location. Other localities more remote from Mexico City showed more damage than was noted here.

LIFE-HISTORY STUDIES

Life-history studies were conducted in a field near the laboratory under conditions as nearly normal as possible. A wire-screen cylinder, 12 inches high and 8 inches in diameter, covered at one end, was inverted over a bean plant on which a pair of beetles had been placed. After an egg mass had been deposited on a leaf, the beetles were moved to another plant. Twelve pairs of beetles were kept under observation in this manner throughout the season. Each cage was observed daily, and records were continued until all the adults of the new generation had emerged.

OVIPOSITION

The first egg mass of the season was obtained the first week of June. The population of beetles in the field continued to increase until late in June, when the maximum number of adults that had passed the dormant period were found. The females continued to oviposit well into September, although by that time they were very much reduced in number. As the season advanced, the intervals between the successive depositions of egg masses became longer. A total of 148 egg masses was deposited, each mass containing from 5 to 60 eggs, the average being 49.73 ± 0.81 eggs.[7]

INCUBATION PERIOD

The length of the incubation period and its relation to temperature are shown in figure 2, in which are plotted the average time required for incubation of varying numbers of egg masses deposited on successive days and the average temperature during the incubation of egg masses deposited each day. For example, the average length of the incubation period of the 7 egg masses deposited on June 29 was 12.6 days, and the average daily mean temperature for this period was 16.9° C.

The incubation period is shown to range in length from 11.1 to 14.6 days. One egg mass, deposited on July 13, had an incubation period of 15 days, and 4 egg masses, deposited on June 23 and 24 and August 15 and 16, had incubation periods of 11 days, but such extremes were reduced by averaging them with records for other egg masses deposited on the same day. The average length of the incubation period for 148 egg masses was 12.94 ± 0.06 days. The average daily mean temperature for the season (June 26 to Sept. 7, inclusive) during which the incubation period was studied was 16.9° C. and the average relative humidity was 72.1 percent.

LARVAL PERIOD

In a similar way the length of the larval period and its relation to temperature are shown in figure 3. The average length of this period ranges from 36 days for eggs hatching on July 7 to 29.2 days for eggs hatching on August 14. The extremes, which are not shown when the average for the larvae in several cages is taken, are 37.2 days for an egg mass hatching July 4 and 28.0 days for an egg mass hatching August 14. The curve showing the length of the larval period has many irregularities that cannot be ascribed to variations in temperature but are probably due to the fact that the larvae may move about and may be shaded more or less by foliage on the plants. This means that the larvae are not always exposed to temperatures recorded on the thermograph. The average length of time spent in this stage of development, as determined from 127 cages containing 2,645 larvae, was 32.79 ± 0.14 days. The average mean daily temperature for the entire larval period, June 26 to September 30, was 17.2° C.; the average relative humidity was 69.7 percent.

[7] Throughout this paper the standard error of the mean is used and not the probable error.

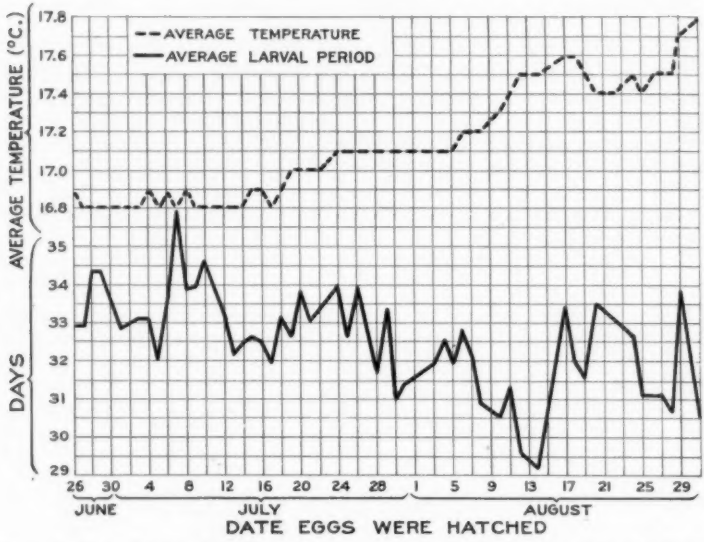

FIGURE 3.—Relation between the length of the larval period of the Mexican bean beetle and the temperature. The lower curve shows the average number of days required for the larval period of individuals from egg masses hatching each day during the season; the upper curve shows the average temperature during each of these periods.

The data concerning the length of time spent in the several instars, the prepupal period, and the total larval period are shown in table 4.

TABLE 4.—*Length of instars and prepupal and larval periods of Epilachna corrupta, San Jacinto, 1930*

Instar and period	Cages	Individuals	Length of period, based on the cage as a unit		
			Maximum	Minimum	Average
	Number	*Number*	*Days*	*Days*	*Days*
Entire larval period	127	2,645	37.2	28	32.79±0.14
First instar	96	2,667	10	6	7.75± .09
Second instar	90	2,463	9	5	6.12± .08
Third instar	81	2,144	9	5	6.71± .12
Fourth instar less prepupal period	70	1,421	11	6.9	8.45± .11
Fourth instar including prepupal period	78	1,605	15.7	9	12.24± .12
Prepupal period	122	2,556	5.3	2.2	3.9± .05

PUPAL PERIOD

The length of the pupal period and its relation to temperature are shown in figure 4. The length of this period ranged from 9 to 12.6 days, but there were extremes of 13 days (individuals entering this period on Aug. 27) and 9 days (individuals entering this period on Sept. 22) when separate cages are taken into account. The average for 119 cages containing 2,090 pupae was 11.33 ± 0.08 days. The average daily mean temperature for the time pupae were studied (July 26 to Oct. 4, inclusive) was 17.4° C.; the average relative humidity, 67.3 percent. Since the pupal period is shorter and the pupae

remain fixed on the under surface of the leaves, the trend in temperature and its relation to the length of the pupal period are shown more clearly than for either the incubation period or the larval period. There are, however, several departures from the general trend that cannot be explained on the basis of temperature.

ENTIRE DEVELOPMENTAL PERIOD

One generation of *Epilachna corrupta* developed at Mexico City (San Jacinto) in an average of 56.74 ± 0.21 days. The extremes were 61.8 and 50 days. These records represent 1,687 beetles contained in 94 cages. The average temperature for the 102-day period from June 26 to October 5, inclusive, was 17.2° C., and the average relative humidity was 66.6 percent. A total of 2,097 adults, including

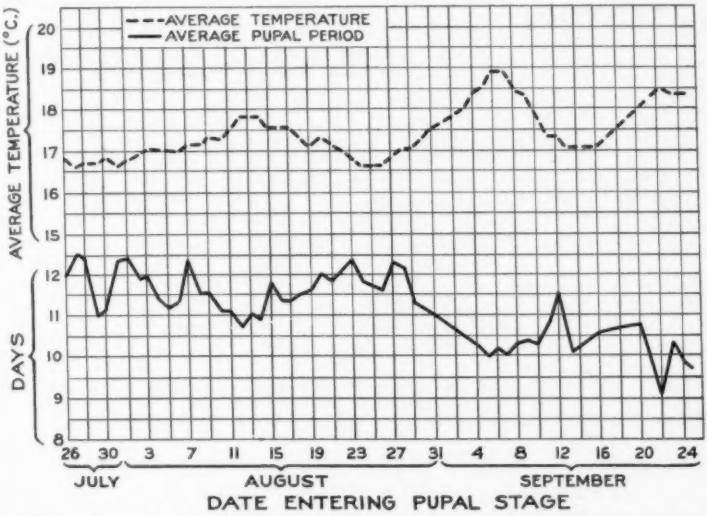

FIGURE 4.—Relationship between the length of the pupal period of the Mexican bean beetle and the temperature. The lower curve shows the average number of days required for the pupal period of larvae entering this stage each day during the season; the upper curve shows the average temperature for this period.

some whose life history was not recorded, emerged during this period. Of these, 975, or 46.5 percent, were males. The first adults of the season appeared July 26, 1930. Marcovitch and Stanley (*5*) determined the length of the developmental period of the bean beetle in the laboratory at four constant temperatures. If a curve is drawn to represent their results at these temperatures (fig. 5), the writers' figure for the total period of development from egg to adult (56 days) will pass through this curve.

BEHAVIOR LATE IN THE SEASON

Seven female and six male beetles that emerged August 8 were placed in a cage for observation. The beetles fed ravenously, but there was no oviposition until August 26, when a mass of 5 eggs was recorded. Four scattered eggs were laid September 5, and a mass of

31 was deposited September 12. The beetles consumed less and less foliage, and in the second week of September they ceased feeding altogether. Thereafter they remained in a state of torpor in protected places on the plant. The beetles were completely bronzed during this period.

OVERWINTERING

Beetles can be found in the fields until the frosts of early October destroy the plants. It is a matter of conjecture where the beetles pass the dry season from October until June. E. G. Smyth [8] reports the natives as saying that swarms of adults were seen to rise from the fields when there was no longer food and that they were carried off by the wind. Alfonso Dampf found an adult bean beetle in wheat stubble on the grounds of the Mexican Department of Agriculture in the winter of 1928.

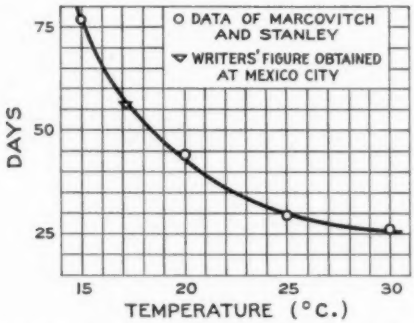

FIGURE. 5.—Relationship between the length of the total developmental period of the Mexican bean beetle at constant temperature as determined by Marcovitch and Stanley (*5*) in Knoxville, Tenn., and also as determined by the present writers under field conditions in San Jacinto, Mexico.

SUMMARY AND CONCLUSIONS

The Mexican bean beetle (*Epilachna corrupta* Muls.), a widely distributed and destructive pest of beans in Mexico, has been recorded from elevations ranging from 3 to 8,845 feet, chiefly within the area delimited by the 20° C. isotherm, which includes most of the central plateau of Mexico. Precipitation and extremes of temperature within this area vary considerably. The effects of these factors on the development of the bean beetle have been determined only in Mexico City.

Severe damage caused by this insect was observed at Atlixco, Puebla (6,171 feet), and at Cuernavaca, Morelos (5,059 feet). In the vicinity of Mexico City the heaviest infestation was found near the villages of Mixquit and Chalco, but less than half of the plants showed any serious injury.

Life-history studies made near Mexico City under field conditions show the length of time spent in each developmental stage throughout the season and its relation to the temperature. One generation of bean beetles matured in an average of 56.74 ± 0.21 days at a mean

[8] Correspondence with N. F. Howard, 1923.

temperature of 17.2° C. and an average relative humidity of 66.6 percent.

Apparently the destruction of the bean plants by frost has much to do with the disappearance of the adult insects from the fields in the fall. In one instance beetles continued to feed and oviposit 4 months beyond the time of their natural occurrence in the field. This was probably due to the favorable temperature and humidity maintained in a protected spot where beans were grown. It is not known where and how the adults pass the dry season in Mexico.

Optimum conditions for the bean beetle are not found in the Valley of Mexico, if we consider temperature, number of generations, and injury done to beans.

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U. S. GOVERNMENT PRINTING OFFICE, 1935

INDEX

Page

Acclimatization Field Station, weather data, 1931–33 436–437
Acetone, effect on curly top virus, experiments 230–232
ACKERT, J. E.; EISENBRANDT, L. L.; WILMOTH, J. H.; GLADING, B.; and PRATT, I.: Comparative Resistance of Five Breeds of Chickens to the Nematode *Ascaridia lineata* (Schneider) 607–624
Agglutinins, appearance in blood of pigs infected with *Erysipelothrix rhusiopathiæ* 71–74
Alcohol, effect on curly top virus, experiments 230–232
ALDRICH, W. W.; WORK, R. A.; and LEWIS, M. R.: Pear Root Concentration in Relation to Soil-Moisture Extraction in Heavy Clay Soil 975–988
Alfalfa—
  cutting practices, effect on trend of organic food reserves in roots. C. O. Grandfield 697–709
  hay, constituents, microbiological decomposition. Max Phillips, M. J. Goss, E. A. Beavens, and L. H. James 761–775
  roots, organic food reserves in, trend as affected by cutting practices. C. O. Grandfield 697–709
Alternaria sp., cultural characteristics 805–806
Alysia—
  fossulata, distribution and habits 481, 484
  ridibunda, distribution, habits, and hosts 481–484
ANDERSON, M. E., and WALKER, J. C.: Histological Studies of Wisconsin Hollander and Wisconsin Ballhead Cabbage in Relation to Resistance to Yellows 823–836
ANDRUS, C. F.; HARTER, L. L.; and ZAUMEYER, W. J.: Studies on Bean Rust Caused by *Uromyces phaseoli typica* 737–759
Aphaereta muscae, parasitism on blowfly pupae, and range 481, 491
Apricot trees, boron injury, histological study 189–193
ARCHIBALD, J. G., and BENNETT, E.: Yield and Chemical Composition of Certain Species of Grass 711–715
ARMSTRONG, M. R.; MACLEOD, F. L.; HEAP, M. E.; and TOLBERT, L. A.: The Vitamin A Content of Five Varieties of Sweetpotato 181–187
ARNOLD, P. T. DIX; BECKER, R. B.; NEAL, W. M.; and SHEALY, A. L.: A Study of the Palatability and Possible Toxicity of 11 Species of *Crotalaria*, Especially of *C. spectabilis* Roth 911–922
Ascaridia lineata—
  hosts, discussion 619–621
  resistance of five breeds of chickens to. J. E. Ackert, L. L. Eisenbrandt, J. H. Wilmoth, B. Glading, and I. Pratt 607–624
Autosyndesis, coefficient of, in progeny of maize-teosinte hybrid, studies 129–131

Bacteria, pathogenic, inducing cell stimulation in plants, physiological studies. J. A. Pinckard 933–952
BAKER, G. O., and VANDECAVEYE, S. C.: Effect of Fertilizers, Soil Type, and Certain Climatic Factors on the Yield and Composition of Oats and Vetch 961–974
BARTHOLOMEW, E. T.; SINCLAIR, W. B.; and NEDVIDEK, R. D.: The Isolation and Distribution of Nitrogen in Dilute Alkali-Soluble Proteins of Healthy Valencia and Washington Navel Orange Fruits 173–180
BARTHOLOMEW, R. P.; JACOB, K. D.; BROWN, B. E.; PIERRE, W. H.; REID, F. R.; and TIDMORE, J. W.: Nutrient Value of the Phosphorus in Calcined Phosphate as Determined by Growth of Plants in Greenhouse Experiments 837–848
Bean beetle, Mexican—
  distribution in Mexico 990–993
  in Mexico. B. J. Landis and C. C. Plummer 989–1001
  life history 996–999
  seasonal activity in Mexico 993–994, 999–1000
Bean rust—
  caused by *Uromyces phaseoli typica*, studies. L. L. Harter, C. F. Andrus, and W. J. Zaumeyer 737–759
  control, experiments 755–757
  infection—
    environmental factors affecting 742–745
    time requirement 746–747
  occurrence and epidemics, relation to weather conditions 748–750
  physiologic forms 752–755
  spore forms, studies 738–742
Beans, varietal susceptibility and resistance to rust 750–752
BEAUMONT, A. B., and SNELL, M. E.: The Effect of Magnesium Deficiency on Crop Plants 553–562
BEAVENS, E. A.; PHILLIPS, MAX; GOSS, M. J.; and JAMES, L. H.: The Microbiological Decomposition of the Constituents of Alfalfa Hay 761–775
BECKER, R. B.; NEAL, W. M.; ARNOLD, P. T. DIX; and SHEALY, A. L.: A Study of the Palatability and Possible Toxicity of 11 Species of *Crotalaria*, Especially of *C. spectabilis* Roth 911–922
Beet pulp—
  analysis, methods 167
  constituents, effects of fermentation on 170–171
  fermentation—
    bacteriological studies 166–167
    efficiency of neutralizing agents 169–170
    influence of hydrogen-ion concentration levels 168–169

Page

Beet pulp—Continued.
fermentation—Continued.
oxygen requirements 167–168
thermophilic fermentation of, O. L. Osburn, J. Stritar, and C. H. Werkman 165–17:
Beggarweed, decomposition rate in Norfolk sand, experiments 717–729
BELL, CHARLES E.: Rate of Decomposition of Organic Matter in Norfolk Sand as Measured by the Formation of Carbon Dioxide and Nitrates 717–730
BENNETT, C. W.: Studies on Properties of the Curly Top Virus 211–241
BENNETT, E., and ARCHIBALD, J. G.: Yield and Chemical Composition of Certain Species of Grass 711–715
Bentgrass, Rhode Island, yield and chemical composition 712–715
BLACK, ALEX; BRAMAN, WINFRED W.; KAHLENBERG, O. J.; VORIS, LEROY; SWIFT, R. W.; and FORBES, E. B.: The Utilization of Energy-Producing Nutriment and Protein in White and Yellow Corn and in Diets Deficient in Vitamins A, D, and G 1–37
BLISS, C. I.; CRESSMAN, A. W.—and BROADBENT, B. M.: Productivity of the Camphor Scale and the Biology of Its Egg and Crawler Stages 243–266
KESSELS, L. T., and DUMESTRE, J. O.: Biology of the Camphor Scale and a Method for Predicting the Time of Appearance of Stages in the Field 267–283
Blowflies—
development in carrion, factors affecting 479–480
parasites of, in North America, R. A. Roberts 479–494
Bluegrass species, yield and chemical composition 712–715
Boron—
injury to leaves and twigs, symptoms 189
toxic concentration, plants grown in, histological characteristics, Irma E. Webber 189–194
BOURNE, B. A.: A Comparative Study of Certain Morphological Characters of Sugarcane × Sorgo Hybrids 539–552
Brachymeria—
compsiluraе—
comparison with *Brachymeria intermedia* 520–521
distribution, hosts, and economic importance 509–511
effectiveness as a parasite 520
immature stages, development 519–520
life history 512–516
seasonal history and habits 517–519
(Cwfd), secondary parasite of gypsy moth, Phillip B. Dowden 495–523
fonscolombei, distribution, habits, and hosts 489–490
intermedia—
comparison with *Brachymeria compsiluraе* 520–521
distribution, hosts, and economic importance 497–499
effectiveness as a parasite 508–509
immature stages, development 508
life history 499–505
(Nees), primary parasite of gypsy moth, Phillip B. Dowden 495–523
seasonal history and habits 505–508

Page

BRAMAN, WINFRED W.; BLACK, ALEX; KAHLENBERG, O. J.; VORIS, LEROY; SWIFT, R. W.; and FORBES, E. B.: The Utilization of Energy-Producing Nutriment and Protein in White and Yellow Corn and in Diets Deficient in Vitamins A, D, and G 1–37
BREWBAKER, H. E., and DEMING, G. W.: Effect of Variations in Stand on Yield and Quality of Sugar Beets Grown under Irrigation 195–210
BROADBENT, B. M.; BLISS, C. I.; and CRESSMAN, A. W.: Productivity of the Camphor Scale and the Biology of Its Egg and Crawler Stages 243–266
Broccoli, absorption of selenium from soils, factors affecting 415
BROWN, B. E.; JACOB, K. D.; BARTHOLOMEW, R. P.; PIERRE, W. H.; REID, F. R.; and TIDMORE, J. W.: Nutrient Value of the Phosphorus in Calcined Phosphate as Determined by Growth of Plants in Greenhouse Experiments 837–848
Buckwheat, chlorosis caused by magnesium deficiency, study 554, 555, 557, 558, 561
Cabbage—
growth, relation to nutrient value of phosphorus on, greenhouse experiments 841–846
root, anatomical studies 825, 829–834
roots, infection with yellows organism, study 825–829
varieties, comparison of susceptibility to yellows infection 825–829
Wisconsin—
Ballhead, histological studies in relation to resistance to yellows, M. E. Anderson and J. C. Walker 823–836
Hollander, histological studies in relation to resistance to yellows, M. E. Anderson and J. C. Walker 823–836
yellows, resistance of Wisconsin Hollander and Wisconsin Ballhead varieties, histological studies, M. E. Anderson and J. C. Walker 823–836
Cadophora—
brunnescens, n. sp., description 803
repens, n. sp., description 803–804
Calculator, electric, use in obtaining multiple correlations, method 59–69
CALDWELL, JOSEPH S.; CULPEPPER, CHARLES W.; and MOON, HUBERT, H.: A Physiological Study of Development and Ripening in the Strawberry 645–696
Calves—
vesicular follicles, development in 953–959
ovarian development, L. E. Casida, A. B. Chapman, and I. W. Rupel 953–960
Camphor scale—
biological studies 243–283
biology, and method for predicting time of appearance of stages in the field, A. W. Cressman, C. I. Bliss, L. T. Kessels, and J. O. Dumestre 267–283
crawler, emergence and settling, study 252–265
development rate, study 269–273
egg stage, biology 248–252
mortality 273, 281–282
productivity, and biology of egg and crawler stages, C. I. Bliss, A. W. Cressman, and B. M. Broadbent 243–266

Page

Camphor scale—Continued.
seasonal history 276–278
settling place, factors affecting 273–274
stages, time of appearance, prediction 274–281
Carbon dioxide—
evolution in Norfolk sand, effect of soil treatments 721–723
formation, effect on rate of decomposition of organic matter in Norfolk sand. Charles E. Bell 717–730
CASIDA, L. E.; CHAPMAN, A. B.; and RUPEL, I. W.: Ovarian Development in Calves 953–960
Cattle, feeding with 11 species of *Crotalaria*, experiments 912–921
Cell stimulation in plants, induced by pathogenic bacteria, physiological studies. J. A. Pinckard 933–952
Ceratostomataceæ, importance as wood-staining fungi 795–802
Ceratostomella—
ips—
cultural characteristics 796–797
occurrence in pine, studies 791–792, 793
multiannulata, n. sp., description 797–798
obscura, n. sp., description 798
pilifera—
cultural characteristics 797
occurrence in pine 791–793
pini, cultural characteristics 798
pluriannulata—
cultural characteristics 798
occurrence in lumber 792–795
Chalcis. See *Brachymeria*.
CHAPMAN, A. B.; CASIDA, L. E.; and RUPEL, I. W.: Ovarian Development in Calves 953–960
Charcoal rot of sweetpotatoes, temperature, wounding, and humidity, studies 316–327
Cherry—
flower buds, winter-killing, effect on yield 469–470
Montmorency—
flower-buds—
delayed winter-killing 565–568
formation, limited, effect on productivity 458–462
injuries, types, correlations 571–572
susceptibility to injury from low temperatures. V. R. Gardner 563–572
leaf-bud production, association with barrenness 462–465
settling of fruit, effect on yield 472–477
unproductive sports, study. V. R. Gardner 457–478
Chick embryo—
mortality caused by malposition. W. Franklin Dove 923–931
positions—
at different ages, classification. W. Franklin Dove 923–931
classification code 925–926
Chickens—
age, effect on resistance to *Ascaridia lineata* 618–619, 621
feeding—
cod-liver oil of various percentages to, effect on vitamin A content of eggs. Grace M. DeVaney, Harry W. Titus, and Ralph B. Nestler 853–860
viosterol to, effect on vitamin A content of eggs. Grace M. DeVaney, Harry W. Titus, and Ralph B. Nestler 853–860

Chickens—Continued.
Page
five breeds, comparative resistance to nematode *Ascaridia lineata* (Schneider). J. E. Ackert, L. L. Eisenbrandt, J. H. Wilmoth, B. Glading, and I. Pratt 607–624
Chicks, hatching failures, relation to position of embryo 929–930
Chlorosis in plants, result of magnesium deficiency, studies 553–554, 557–561
CHRISTENSEN, J. J., and JOHNSON, I. J.: Field Reaction of Varieties and Selfed Lines of Corn to Different Collections of *Ustilago zeae* 47–57
Citrus, nitrogen in, isolation and distribution of, studies 173–179
Claviceps purpurea, factor in nutritive value of rye for rats and swine. D. W. Johnson, and L. S. Palmer 39–45
Climate—
effect on yield and composition of oats and vetch. G. O. Baker and S. C. Vandecaveye 961–974
of Mexico City, relation to Mexican bean beetle infestations 994–995
Clover, white—
Dutch, yield and chemical composition 712–715
nutrients, digestibility and availability, effect of artificial drying on 150–163
Cod-liver oil, feeding to chickens, effect on vitamin A content of eggs. Grace M. DeVaney, Harry W. Titus, and Ralph B. Nestler 853–860
Cold resistance, inheritance in winter wheat, with preliminary studies on the technic of artificial freezing tests. W. W. Worzella 625–635
COLE, J. R.: *Gnomonia nervisedа*, the Perfect Stage of the Fungus that Causes the Vein Spot Disease of Pecan Foliage 91–96
COLLINS, G. N., and LONGLEY, A. E.: A Tetraploid Hybrid of Maize and Perennial Teosinte 123–133
Colorado, sugar beets, stand variations, effect on yield and quality 195–209
Compsilura concinnata, parasitization by *Brachymeria compsilurae* 517–518, 520
Corn—
absorption of selenium from soils, factors affecting 413–415
digestibility by pigs, effect of grinding, study 731–735
grinding, effect on digestibility by pigs and on its content of metabolizable energy. W. P. Garrigus and H. H. Mitchell 731–735
magnesium-deficiency studies 553–562
metabolizable energy content, effect of grinding on, study 731–735
nutritive value, comparison of white and yellow 1–36
reactions to smut, experiments 47–56
smut—
collections, field reaction of varieties and selfed lines of corn to 47–56
galls, location, number, and size per plant, study 52–54
varieties and selfed lines, field reaction to collections of *Ustilago zeae*. J. J. Christensen and I. M. Johnson 47–57

Page

Corn—Continued.
white and yellow, energy-producing nutriment and protein in, utilization. Winfred W. Braman, Alex Black, O. J. Kahlenberg, LeRoy Voris, R. W. Swift, and E. B. Forbes 1–37
See also Maize.
Correlation coefficients, multiple, rapid calculation of, procedure for. G. N. Stroman 59–69
Cotton—
boll, age, effect on germination of seed, study 433–434
varieties, boll opening—
dormancy of seed during 429–432
period, seed moisture content and germination during 435–442
Cottonseed—
deterioration in—
field, study 443–446
storage, study 449–456
dormancy and maturity. D. M. Simpson 429–434
germination, effect of—
age 433–434
moisture content in different types of storage 453–456
maturity and dormancy. D. M. Simpson 429–434
moisture content and germination during boll-opening period 435–442
raw, feeding to rats, experiments 899–908
stored, deterioration, relation to moisture content and storage method. D. M. Simpson 449–456
viability, effect of field conditions. D. M. Simpson and B. M. Stone 435–447
Cowpea—
decomposition rate in Norfolk sand, experiments 717–729
rust, comparison with bean rust 741–742
CREECH, G. T., and SCHOENING, H. W.: Serological Studies of Swine Erysipelas with Particular Reference to Agglutination 71–79
CRESSMAN, A. W.; BLISS, C. I.—and BROADBENT, B. M.: Productivity of the Camphor Scale and the Biology of Its Egg and Crawler Stages 243–266
KESSELS, L. T.; and DUMESTRE, J. O.: Biology of the Camphor Scale and a Method for Predicting the Time of Appearance of Stages in the Field 267–283
Crop—
plants, effect of magnesium deficiency on. A. B. Beaumont and M. E. Snell 553–562
yields, relation to magnesium sulphate and lime content in soil, experiments 555–556
Crossbreeding, sugarcane and sorgo, experiments 541–551
Crotalaria—
decomposition rate in Norfolk sand, experiments 717–729
11 species, palatability and possible toxicity, study. R. B. Becker, W. M. Neal, P. T. Dix Arnold, and A. L. Shealy 911–922
spectabilis Roth, palatability and possible toxicity, study. R. B. Becker, W. M. Neal, P. T. Dix Arnold, and A. L. Shealy 911–922
CULPEPPER, CHARLES W.; CALDWELL, JOSEPH S.; and MOON, HUBERT H.: A Physiological Study of Development and Ripening in the Strawberry 645–696

Page

Curly-top virus—
dilution and filtration tests 220–223
effect of hydrogen-ion concentration, experiments 229–230, 237
inactivation by plant juices, tests 233–236, 238
longevity, effect of desiccation on, experiments 224–226
properties, studies. C. W. Bennett 211–241
removal by precipitation and purification, experiments 217–219
resistance to aging in liquid medium, tests 223–224
Cytogenetics of tetraploid maize. L. F. Randolph 591–605

DAVIDSON, ROSS W.: Fungi Causing Stain in Logs and Lumber in the Southern States, Including Five New Species 789–807
DEMING, G. W., and BREWBAKER, H. E.: Effect of Variations in Stand on Yield and Quality of Sugar Beets Grown under Irrigation 195–210
Desmodium tortuosum, decomposition rate in Norfolk sand, experiments 717–729
DEVANEY, GRACE M.; TITUS, HARRY W.; and NESTLER, RALPH B.: Vitamin A Content of Eggs Produced by Chickens Fed Viosterol and Various Percentages of Cod-Liver Oil 853–860
Diamond-skin disease, of pigs, agglutination tests 74–75
DICKE, F. F., and PHILLIPS, W. J.: Morphology and Biology of the Wheat Jointworm Gall 359–386
Dictyostelium discoideum—
a new species of slime mold from decaying forest leaves. Kenneth P. Raper 135–147
aggregation stage, description 139–140
isolation and culture 136–137
migration stage, description 140–142
n. sp., technical description 135–136
sorocarp formation, description 142–147
vegetative stage, description 137–138
Diets deficient in vitamins A, D, and G, utilization of energy-producing nutriment and protein in. Winfred W. Braman, Alex Black, O. J. Kahlenberg, LeRoy Voris, R. W. Swift, and E. B. Forbes 1–37
Diplodia—
megalospora, cultural characteristics 803
natalensis, cultural characteristics 803
tubericola, infection of sweetpotatoes, factors affecting 306–316, 325–327
DOVE, W. FRANKLIN: Classification of Chick-Embryo Positions at Different Ages and Malposition as a Cause of Mortality 923–931
DOWDEN, PHILLIP B.: *Brachymeria intermedia* (Nees), Primary Parasite, and *B. compsilurae* (Cwfd), Secondary Parasite, of Gypsy Moth 495–523
Drying, artificial, temperature of, effect on digestibility and availability of nutrients in pasture herbage. R. E. Hodgson, J. C. Knott, R. R. Graves, and H. K. Murer 149–164
DUMESTRE, J. O.; CRESSMAN, A. W.; BLISS, C. I.; and KESSELS, L. T.: Biology of the Camphor Scale and a Method for Predicting the Time of Appearance of Stages in the Field 267–283

	Page
Egg production, correlation studies, and possible genetic interpretations. Charles W. Knox, Morley A. Jull, and Joseph P. Quinn	573–589
Eggs—	
hatching date, relation to egg production	573, 575–577
vitamin A content, production by feeding chickens viosterol and percentages of cod-liver oil. Grace M. DeVaney, Harry W. Titus, and Ralph B. Nestler	853–860
Eisenbrandt, L. L.; Ackert, J. E.; Wilmoth, J. H.; Glading, B.; and Pratt, I.: Comparative Resistance of Five Breeds of Chickens to the Nematode *Ascaridia lineata* (Schneider)	607–624
Endoconidiophora—	
adiposa, n. comb., description	802
coerulescens—	
cultural characteristics	798–800
occurrence in lumber	792, 794–795
fimbriata, n. comb., description	800–801
moniliformis—	
n. comb., description	800
occurrence in lumber	792–795
paradoxa, n. comb., description	802
Energy, metabolizable, content of ground corn, experiments with pigs	731–735
Eniaca texana, parasitism on blowfly larvae	490–491
Epilachna corrupta. *See* Bean beetle, Mexican.	
Ergot, factor in nutritive value of rye for rats and swine. D. W. Johnson and L. S. Palmer	39–45
Erysipelas, swine—	
serological studies with particular reference to agglutination. H. W. Schoening and G. T. Creech	71–79
urticarial form, agglutination test	74–75
Erysipelothrix rhusiopathiae, cultures, identification method	77–78
Euchlaena perennis, tetraploid hybrid of	123–133
Eutettix tenellus, agent in spread of curly top virus, study	212–213, 219–222
Fermentation, thermophilic, of beet pulp. O. L. Osborn, J. Stritar, and C. H. Werkman	165–172
Fertilizers, effect upon—	
chlorosis in plants, studies	553–556
yield and composition of oats and vetch. G. O. Baker and S. C. Vandecaveye	961–974
Fescue, sheep, yield and chemical composition	712–715
Fires, grass, annual, effect on organic matter and other constituents of virgin longleaf pine soils. S. W. Greene	809–822
Flour beetle, vitamin D need and phosphorus content and requirements, study. J. W. Nelson and L. S. Palmer	849–852
Fluorine, volatilization, relation to nutrient value of calcined phosphate	845–846
Forage—	
crops, drying, artificial, effect on nutritive value, studies	149–163
growth on burned and unburned areas, experiments	814–816
Forbes, E. B.; Braman, Winfred W.; Black, Alex; Kahlenberg, O. J.; Voris, LeRoy; and Swift, R. W.: The Utilization of Energy-Producing Nutriment and Protein in White and Yellow Corn and in Diets Deficient in Vitamins A, D, and G	1–37
Forest leaves, decaying, *Dictyostelium discoideum*, a new species of slime mold from. Kenneth B. Raper	135–147
Fragaria spp. *See* Strawberry.	
Freezing tests, artificial, technic, studies	627–632
Frost, effect on—	
flower buds of Montmorency cherry	568–571
yield of Montmorency cherry	470–472
Fruit trees, boron injury, histological study	189–193
Fungi—	
blue-staining, occurrence in pine timber	789, 791–795
five new species causing stain in logs and lumber in Southern States. Ross W. Davidson	789–807
Imperfecti, importance as wood-staining fungi	802–806
storage rot, infection and decay of sweetpotatoes, factors affecting. J. I. Lauritzen	285–329
wood-staining—	
cultural characteristics	795–806
in Southern States, studies	789–806
new species, association with *Scolytus ventralis*. Ernest Wright	525–538
Fusarium—	
conglutinans, cause of cabbage yellows	823
end rot, of sweetpotatoes, factors affecting	303–306, 324–327
oxysporum, infection of sweetpotatoes, factors affecting	301–303, 324–327
Gall-forming bacteria, on plants, physiological studies	933–951
Gardner, V. R.—	
A Study of Some Unproductive Sports of the Montmorency Cherry	457–478
The Susceptibility of Flower Buds of the Montmorency Cherry to Injury from Low Temperature	563–572
Garrigus, W. P., and Mitchell, H. H.: The Effect of Grinding on the Digestibility of Corn by Pigs and on Its Content of Metabolizable Energy	731–735
Germicides, effect on sugar beet curly top virus, experiments	232–233
Gersdorff, W. A.—	
A New Criterion for the Comparison of Toxicity with Respect to Concentration and Time	881–891
The Quantitative Relationship between the Constitution and Toxicity of Some Rotenone Derivatives	893–898
Glading, B.; Ackert, J. E.; Eisenbrandt, L. L.; Wilmoth, J. H.; and Pratt, I.: Comparative Resistance of Five Breeds of Chickens to the Nematode *Ascaridia lineata* (Schneider)	607–624
Gnomonia—	
caryae var. *pecanae*, n. var., comparison with *G. nervisedal nerviseda*—	96
n. comb.—	
comparison with *G. caryae* var. *pecanae*	96
technical description	92–93
perfect stage of fungus causing vein spot disease of pecan foliage. J. R. Cole	91–96
Goss, M. J.; Phillips, Max; Beavens, E. A.; and James, L. H.: The Microbiological Decomposition of the Constituents of Alfalfa Hay	761–775

Page

Gossypol—
effect upon muscle of small intestine of rat. L. A. Moore 899–909
feeding to rats, experiments 899–908
Grain yield among 1,500 wheat nursery plots, variation and correlation. Gustav A. Wiebe 331–357
GRANDFIELD, C. O.: The Trend of Organic Food Reserves in Alfalfa Roots as Affected by Cutting Practices 697–709
Grape leaves, boron injury, histological study 189–190, 192–193
Graphium rigidum—
cultural characteristics 805
occurrence in lumber 792, 794–795
Grass—
species, chemical composition and yield. J. G. Archibald and E. Bennett 711–715
winter burning, effect on soils, investigations 809–821
GRAVES, R. R.; HODGSON, R. E.; KNOTT, J. C.; and MURER, H. K.: Effect of Temperature of Artificial Drying on Digestibility and Availability of Nutrients in Pasture Herbage 149–164
GREENE, S. W.: Effect of Annual Grass Fires on Organic Matter and Other Constituents of Virgin Longleaf Pine Soils 809–822
Gypsy moth—
primary parasite of, *Brachymeria intermedia* (Nees.) Phillip B. Dowden 495–523
secondary parasite of, *Brachymeria compsilurae* (Cwfd.). Phillip B. Dowden 495–523

Hardwood, infestation by blue-stain fungi, study 794–795
Harmolita tritici—
cause of wheat-stem galls 359
oviposition, description 360–369
See also Wheat jointworm.
Harmonema pullulans, cultural characteristics 805
HARTER, L. L.; ANDRUS, C. F.; and ZAUMEYER, W. J.: Studies on Bean Rust Caused by *Uromyces phaseoli typica* 737–759
Hay, alfalfa, constituents, microbiological decomposition. Max Phillips, M. J. Goss, E. A. Beavens, and L. H. James 761–775
HEAP, M. E.; MACLEOD, F. L.; ARMSTRONG, M. R.; and TOLBERT, L. A.: The Vitamin A Content of Five Varieties of Sweetpotato 181–187
Helminthosporium—
geniculatum, cultural characteristics 805
oryzae—
cause of leaf spot of rice 81
infection of rice leaves, histological study. E. C. Tullis 81–90
Hens—
egg laying, correlation studies 573–588
See also Pullets.
Hicoria pecan. *See* Pecan.
HODGSON, R. E.; KNOTT, J. C.; GRAVES, R. R.; and MURER, H. K.: Effect of Temperature of Artificial Drying on Digestibility and Availability of Nutrients in Pasture Herbage 149–164
Holcus sorghum var. *saccharatus*. *See* Sorgo.
Humidity, effect on—
infection and decay of sweetpotatoes, studies 285–327
viability of stored cottonseed, study 449–456
HURD-KARRER, ANNIE M.: Factors Affecting the Absorption of Selenium from Soils by Plants 413–427

Page

Hybrids, sugarcane × sorgo, morphological characters, comparative studies. B. A. Bourne 539–552
Hydrogen-ion concentration, effect on curly-top virus, experiments 229–230, 237

Ignition, spontaneous, in alfalfa hay, studies 761–773
Inheritance of cold resistance in winter wheat, with preliminary studies on technic of artificial freezing tests. W. W. Worzella 625–635
Intestine, small, of rat, muscular reaction to gossypol. L. A. Moore 899–909
Irrigation—
sugar beets, effect of variations in stand on yield and quality. H. E. Brewbaker and G. W. Deming 195–210
water, boron content, injury to fruit, study 189–193

JACOB, K. D.; BARTHOLOMEW, R. P.; BROWN, B. E.; PIERRE, W. H.; REID, F. R.; and TIDMORE, J. W.: Nutrient Value of the Phosphorus in Calcined Phosphate as Determined by Growth of Plants in Greenhouse Experiments 837–848
JAMES, L. H.; PHILLIPS, MAX; GOSS, M. J.; and BEAVENS, E. A.: The Microbiological Decomposition of the Constituents of Alfalfa Hay 761–775
Java black rot, of sweetpotatoes, factors affecting 306–316, 325–327
JOHNSON, D. W., and PALMER, L. S.: Ergot as a Factor in the Nutritive Value of Rye for Rats and Swine 39–45
JOHNSON, I. J., and CHRISTENSEN, J. J.: Field Reactions of Varieties and Selfed Lines of Corn to Different Collections of *Ustilago zeae* 47–57
JULL, MORLEY A.; KNOX, CHARLES W.; and QUINN, JOSEPH P.: Correlation Studies of Egg Production and Possible Genetic Interpretations 573–589

KAHLENBERG, O. J.; BRAMAN, WINFRED W.; BLACK, ALEX; VORIS, LEROY; SWIFT, R. W.; and FORBES, E. B.: The Utilization of Energy-Producing Nutriment and Protein in White and Yellow Corn and in Diets Deficient in Vitamins A, D, and G 1–37
KESSELS, I. T.; CRESSMAN, A. W.; BLISS, C. I.; and DUMESTRE, J. O.: Biology of the Camphor Scale and a Method for Predicting the Time of Appearance of Stages in the Field 267–283
KNOTT, J. C.; HODGSON, R. E.; GRAVES, R. R.; and MURER, H. K.: Effect of Temperature of Artificial Drying on Digestibility and Availability of Nutrients in Pasture Herbage 149–164
KNOX, CHARLES W.; JULL, MORLEY A.; and QUINN, JOSEPH P.: Correlation Studies of Egg Production and Possible Genetic Interpretations 573–589

LANDIS, B. J., and PLUMMER, C. C.: The Mexican Bean Beetle in Mexico 989–1001
LAURITZEN, J. I.: Factors Affecting Infection and Decay of Sweetpotatoes by Certain Storage Rot Fungi 285–329

Page

Leaf area, rapid measurement, photoelectric device for. Robert B. Withrow 637–643
Legumes, growth on grazed and ungrazed area 815–816
Lemon leaves, boron injury, histological study 189–190, 192–193
Leptographium microsporum, n. sp., description 805
LEWIS, M. R.; ALDRICH, W. W.; and WORK, R. A.: Pear Root Concentration in Relation to Soil-Moisture Extraction in Heavy Clay Soil 975–988
LEXEN, B. R.: Some Factors Influencing the Yield and Mortality of Ponderosa Pine in the Southwest 777–787
Light, effect on—
development of uredial stage of *Puccinia graminis*. Leonard W. Melander 861–880
infection of beans with rust, experiments 745
Lignin, role in spontaneous ignition of hay 772–773
Logs, infestation and staining by fungi, including five new species, in Southern States. Ross W. Davidson 789–807
LONGLEY, A. E., and COLLINS, G. N.: A Tetraploid Hybrid of Maize and Perennial Teosinte 123–133
Lumber, infestation and staining by fungi, including five new species in Southern States. Ross W. Davidson 789–807

MACLEOD, F. L.; ARMSTRONG, M. R.; HEAP, M. E.; and TOLBERT, L. A.: The Vitamin A Content of Five Varieties of Sweetpotato 181–187
Magnesium deficiency, effect on crop plants. A. B. Beaumont and M. E. Snell 553–562
Maize—
and perennial teosinte, tetraploid hybrid of. G. N. Collins and A. E. Longley 123–133
teosinte—
homologous chromosomes, synaptic attraction 123–133
hybrid—
autosyndesis, measures of progeny, coefficient of autosyndesis in, studies 124–125, 129–131
tetraploid hybrid, description and progeny 125–127
tetraploid—
and diploid intercrosses 596–603
appearance and breeding behavior 591–595
chromosome behavior 603–604
comparison with diploid 591–605
cytogenetics. L. F. Randolph 591–605
hybrids, studies 123–133, 591–603
See also Corn.
Mangels, chlorosis caused by magnesium deficiency, study 554, 555, 558
Manure, decomposition rate in Norfolk sand, experiments 717–729
Medicago sativa. *See* Alfalfa.
MELANDER, LEONARD W.: Effect of Temperature and Light on Development of the Uredial Stage of *Puccinia graminis* 861–880
Metabolism, pigs, effect of ground and whole corn on, comparative tests 731–735
Mexican bean beetle. *See* Bean beetle, Mexican.
Mexico City, climate, relation to Mexican bean beetle infestations 994–995

Page

Mexico, Mexican bean beetle in. B. J. Landis and C. C. Plummer 989–1001
Micro-organisms, relation to decomposition of constituents of alfalfa hay 761–775
Millet—
absorption of selenium from soils, factors affecting 413–415
growth, relation to nutrient value of phosphorus on, greenhouse experiments 841–846
MITCHELL, H. H., and GARRIGUS, W. P.: The Effect of Grinding on the Digestibility of Corn by Pigs and on Its Content of Metabolizable Energy 731–735
MOON, HUBERT H.; CULPEPPER, CHARLES W.; and CALDWELL, JOSEPH S.: A Physiological Study of Development and Ripening in the Strawberry 645–696
MOORE, L. A.: Effect of Gossypol upon the Muscle of the Small Intestine of the Rat 899–909
Mormoniella vitripennis, parasitism on blowfly pupae 481, 491–492
MURER, H. K.; HODGSON, R. E.; KNOTT, J. C.; and GRAVES, R. R.: Effect of Temperature of Artificial Drying on Digestibility and Availability of Nutrients in Pasture Herbage 149–164
MURPHY, H. C.: Effect of Crown Rust Infection on Yield and Water Requirement of Oats 387–411
Muscle, intestinal, of rat, effect of gossypol upon. L. A. Moore 899–909
Mustard, absorption of selenium from soils, factors affecting 415

Natal grass, decomposition rate in Norfolk sand, experiments 717–729
NEAL, W. M.; BECKER, R. B.; ARNOLD, P. T. DIX; and SUESLY, A. L.: A Study of the Palatability and Possible Toxicity of 11 Species of *Crotalaria*, Especially of *C. spectabilis* Roth 911–922
NEDVIDEK, R. D.; SINCLAIR, W. B.; and BARTHOLOMEW, E. T.: The Isolation and Distribution of Nitrogen in Dilute Alkali-Soluble Proteins of Healthy Valencia and Washington Navel Orange Fruits 173–180
NELSON, J. W., and PALMER, L. S.: The Phosphorus Content and Requirements of the Flour Beetle *Tribolium confusum* Duval, and a Study of Its Need for Vitamin D 849–852
Nematode, infestation of chickens, study 607–622
NESTLER, RALPH B.; DEVANEY, GRACE M.; and TITUS, HARRY W.: Vitamin A Content of Eggs Produced by Chickens Fed Viosterol and Various Percentages of Cod-Liver Oil 853–860
Nitrates—
accumulation in Norfolk sand, effect of soil treatments on 725–729
formation, effect on rate of decomposition of organic matter in Norfolk sand. Charles E. Bell 717–730
Nitrogen, isolation and distribution of in dilute alkali-soluble proteins of healthy Valencia and Washington navel orange fruits. W. B. Sinclair, E. T. Bartholomew, and R. D. Nedvidek 173–180
Norfolk sand, organic matter in, decomposition rate as measured by the formation of carbon dioxide and nitrates. Charles E. Bell 717–730

Nutrients, in pasture herbage, digestibility and availability of, effect of temperature of artificial drying on. R. E. Hodgson, J. C. Knott, R. R. Graves, and H. K. Murer ... Page 149–164

Nutrition, swine, effect of rye containing ergot. D. W. Johnson and L. S. Palmer ... 39–45

Oat stem rust, development studies ... 861–879

Oats—
- composition, effect of fertilizers, soil type, and certain climatic factors on. G. O. Baker and S. C. Vandecaveye ... 961–974
- crown-rust infection, effect on yield and water requirement. H. C. Murphy ... 387–411
- field-grown, yield, effect of crown-rust infection on, experiments ... 388–391
- greenhouse-grown, water requirement and yield, effect of crown-rust on ... 391–405
- water requirement, effect of crown-rust infection on. H. G. Murphy ... 387–411
- yield, effect of—
  - crown-rust infection on. H. C. Murphy ... 387–411
  - fertilizers, soil type, and certain climatic factors on. G. O. Baker and S. C. Vandecaveye ... 961–974

Orange fruits, healthy Valencia and Washington navel, isolation and distribution of nitrogen in dilute alkali-soluble proteins of. W. B. Sinclair, E. T. Bartholomew, and R. D. Nedvidek ... 173–180

Oranges, protein, nitrogen in, isolation and distribution, studies ... 173–179

Orchard grass, yield and chemical composition ... 712–715

Osburn, O. L.; Stritar, J.; and Werkman, C. H.: The Thermophilic Fermentation of Beet Pulp ... 165–172

Ovaries, development in calves. L. E. Casida, A. B. Chapman, and I. W. Rupel ... 953–960

Palmer, L. S., and—
- Johnson, D. W.: Ergot as a Factor in the Nutritive Value of Rye for Rats and Swine ... 39–45
- Nelson, J. W.: The Phosphorus Content and Requirements of the Flour Beetle *Tribolium confusum* Duval, and a Study of Its Need for Vitamin D ... 849–852

Parasites of blowflies in North America. R. A. Roberts ... 479–494

Pasture herbage, nutrients in, digestibility and availability of, effect of temperature of artificial drying on. R. E. Hodgson, J. C. Knott, R. R. Graves, and H. K. Murer ... 149–164

Peach stems, boron injury, histological study ... 189–190, 191–193

Pear—
- root concentration, relation to soil-moisture extraction in heavy clay soil. W. W. Aldrich, R. A. Work, and M. R. Lewis ... 975–988
- roots—
  - distribution, relation to soil-moisture extraction, experiments ... 979–986
  - extraction of soil moisture, relation to depth, experiments ... 975–979

Pecan— Page
- composition during fruit development, seasonal changes in, physiological study. Clifford J. B. Thor and Charles L. Smith ... 97–121
- foliage, vein spot disease of fungus causing, perfect stage, *Gnomonia nervisceda*. J. R. Cole ... 91–96
- fruit—
  - ash content, changes, analytical data ... 101, 104–106, 116, 117
  - development, seasonal changes in composition, physiological study. Clifford J. B. Thor and Charles L. Smith ... 97–121
  - dry matter content, determination ... 100, 102–104, 116, 117
  - nitrogen content, changes, analytical data ... 101, 106–108, 116–118
  - oil content, changes, analytical data ... 101, 107, 110, 117, 118
  - polysaccharide content, changes, analytical data ... 101, 113–115, 117
  - protein content, discussion ... 117, 118
  - sugar content, changes, analytical data ... 101, 108–115, 117, 118
- leaves, inoculation with conidia and ascospores of vein spot fungus ... 94
- vein spot—
  - disease, symptoms ... 91–92
  - pathogene, stages, description, and pathogenicity ... 92–96

Pecans, infection with *Gnomonia nervisceda*, description ... 91–92

Penicillium, infection of sweetpotatoes, factors affecting ... 298–301, 304–306, 326, 327

Phillips, Max; Goss, M. J.; Beavens, E. A.; and James, L. H.: The Microbiological Decomposition of the Constituents of Alfalfa Hay ... 761–775

Phillips, W. J., and Dicke, F. F.: Morphology and Biology of the Wheat Jointworm Gall ... 359–386

Phosphate, calcined, nutrient value—
- of phosphorus in as determined by growth of plants in greenhouse experiments. K. D. Jacob, R. P. Bartholomew, B. E. Brown, W. H. Pierre, F. R. Reid, and J. W. Tidmore ... 837–848
- relation to volatilization of fluorine, comparison ... 845–846

Phosphates—
- effect on dry weight and phosphorous content of plants, studies ... 841–843
- efficiencies and availability, comparative data ... 844–845

Phosphorus—
- nutrient value of, in calcined phosphate as determined by growth of plants in greenhouse experiments. K. D. Jacob, R. P. Bartholomew, B. E. Brown, W. H. Pierre, F. R. Reid, and J. W. Tidmore ... 837–848
- requirements of *Tribolium confusum*, study ... 849–852

Photoelectric device for the rapid measurement of leaf area. Robert B. Withrow ... 637–643

Phytomonas—
- *beticola*, cause of—
  - bacterial pocket rot of sugar beets, physiological studies ... 933–951
  - beet galls, physiological studies ... 933–951

Phytomonas—Continued. Page
savastanoi—
cause of olive galls, physiological studies 933–951
var. *nerii*, cause of oleander galls, physiological studies 933–951
tumefaciens, cause of crown galls of plants, physiological studies 933–951

PIERRE, W. H.; JACOB, K. D.; BARTHOLOMEW, R. P.; BROWN, B. E.; REID, F. R.; and TIDMORE, J. W.: Nutrient Value of the Phosphorus in Calcined Phosphate as Determined by Growth of Plants in Greenhouse Experiments 837–848

Pigs—
feeding ground corn, experiments 731–735
inoculation with anti-swine-erysipelas serum, results 75–76
See also Swine.

PINCKARD, J. A.: Physiological Studies of Several Pathogenic Bacteria That Induce Cell Stimulation in Plants 933–952

Pine—
lumber, blue-stain in, investigations 789, 790–795
ponderosa, mortality—
analysis 783–787
and yield in Southwest, factors influencing. B. R. Lexen 777–787
soils, organic matter and other constituents of, effect of annual grass fires on. S. W. Greene 809–822

Pinus ponderosa. *See* Pine, ponderosa.

Plant growth, relation to nutrient value of phosphorus in calcined phosphate, greenhouse experiments. K. D. Jacob, R. P. Bartholomew, B. E. Brown, W. H. Pierre, F. R. Reid, and J. W. Tidmore 837–848

Plants—
absorption of selenium from soils, factors affecting. Annie M. Hurd-Karrer 413–427
cell stimulation in, induced by pathogenic bacteria, physiological studies. J. A. Pinckard 933–952
chemical composition, effect of magnesium deficiency on 560–561
chlorosis caused by magnesium deficiency, studies 553–561
crop, effect of magnesium deficiency on. A. B. Beaumont and M. E. Snell 553–562
grown in toxic concentrations of boron, histological characteristics. Irma E. Webber 189–194
weight and phosphorous content, effect of phosphates on, studies 841–843

PLUMMER, C. C., and LANDIS, B. J.: The Mexican Bean Beetle in Mexico 989–1001

Porthetria dispar. *See* Gypsy moth.

PRATT, I.; ACKERT, J. E.; EISENBRANDT, L. L.; WILMOTH, J. H.; and GLADING, B.: Comparative Resistance of Five Breeds of Chickens to the Nematode *Ascaridia lineata* (Schneider) 607–624

Protein in oranges—
isolation method 174–175
nitrogen distribution 175–176

Proteins, dilute alkali-soluble, of healthy Valencia and Washington navel orange fruits, isolation and distribution of nitrogen in. W. B. Sinclair, E. T. Bartholomew, and R. D. Nedvidek 173–180

Prune trees, boron injury, histological study Page 189–193

Pseudaonidia duplex. *See* Camphor scale.

Psilodora sp., distribution, habits, development, and hosts 485–487

Puccinia—
coronata avenae, infection, effect on yield and water requirement of oats 387–411
graminis, uredial stage, development, effect of temperature and light on. Leonard W. Melander 861–880

Pullets—
broodiness, relation to egg production 585–586
egg laying, correlation studies 573–588
sexual maturity, relation to egg production 578–579

QUINN, JOSEPH P.; KNOX, CHARLES W.; and JULL, MORLEY A.: Correlation Studies of Egg Production and Possible Genetic Interpretations 573–589

RANDOLPH, L. F.: Cytogenetics of Tetraploid Maize 591–605

RAPER, KENNETH B.: *Dictyostelium discoideum*, a New Species of Slime Mold from Decaying Forest Leaves 135–147

Raspberry cane gall, physiological studies 933–951

Rat, intestinal muscle of, effect of gossypol upon. L. A. Moore 899–909

Rations, corn, energy, and protein utilization by rats, experiments 1–36

Rats—
feeding rye containing ergot, effect, experiments. D. W. Johnson and L. S. Palmer 39–45
growth, effect of ergot upon, experiments 39–40, 41–42
utilization of energy-producing nutriment and protein in white and yellow corn, experiments 1–36

Redtop, yield and chemical composition, experiments 712–715

Regression coefficients, partial, calculation method 68–69

REID, F. R.; JACOB, K. D.; BARTHOLOMEW, R. P.; BROWN, B. E.; PIERRE, W. H.; and TIDMORE, J. W.: Nutrient Value of the Phosphorus in Calcined Phosphate as Determined by Growth of Plants in Greenhouse Experiments 837–848

Rhizopus—
infection and decay of sweetpotatoes, factors affecting 285, 287–298, 324–327
soft rot, of sweetpotatoes, factors affecting, studies 285, 287–298, 324–327

Rice leaves—
anatomy 83–89
infection with *Helminthosporium oryzae*, histological studies. E. C. Tullis 81–90

Rickets, in rats, effects of white and yellow corn, experiments 19–21, 24

ROBERTS, R. A.: Some North American Parasites of Blowflies 479–494

Rotenone—
compounds, toxicity—
effect of changes in constitution on 897
new criterion for comparison with respect to concentration and time. W. H. Gersdorff 881–888

Rotenone—Continued. Page
derivatives, constitution and toxicity, quantitative relationship between. W. A. Gersdorff 893–898
toxicity, comparison, new criterion for with respect to concentration and time. W. A. Gersdorff 881–891
RUPEL, I. W.; CASIDA, L. E.; and CHAPMAN, A. B.: Ovarian Development in Calves 953–960
Rye—
infected with ergot, nutritive value for rats and swine. D. W. Johnson and L. S. Palmer 39–45
stem rust, development, studies 861–879
Ryegrass, nutrients, digestibility and avai'ability, effect of artifical drying on 150–163

Sand, Norfolk, decomposition rate of organic matter in, as measured by formation of carbon dioxide and nitrates. Charles E. Bell 717–739
Scale, camphor. *See* Camphor scale.
SCHOENING, H. W., and CREECH, G. T.: Serological Studies of Swine Erysipelas with Particular Reference to Agglutination 71–79
Sclerotium bataticola, infection of sweetpotatoes, factors affecting 316–327
Scolytus—
beetles, carriers of *Trichosporium symbioticum* 531–532
ventralis, association with *Trichosporium symbioticum*, n. sp., wood-staining fungus. Ernest Wright 525–538
Selenium—
absorption from soils by plants, factors affecting. Annie M. Hurd-Karrer 413–423
toxicity to wheat plants, factors affecting 413–426
SHEALY, A. L.; BECKER, R. B.; NEAL, W. M.; and ARNOLD, P. T. DIX: A Study of the Palatability and Possible Toxicity of 11 Species of *Crotalaria*, Especially of *C. spectabilis* Roth 911–922
SIMPSON, D. M.—
Dormancy and Maturity of Cottonseed 429–434
Relation of Moisture Content and Method of Storage to Deterioration of Stored Cottonseed 449–456
and STONE, B. M.: Viability of Cottonseed as Affected by Field Conditions 435–447
SINCLAIR, W. B.; BARTHOLOMEW, E. T.; and NEDVIDEK, R. D.: The Isolation and Distribution of Nitrogen in Dilute Alkali-Soluble Proteins of Healthy Valencia and Washington Navel Orange Fruits 173–180
Slime mold, *Dictyostelium discoideum*, a new species from decaying forest leaves. Kenneth B. Raper 135–147
SMITH, CHARLES L., and THOR, CLIFFORD, J. B.: A Physiological Study of Seasonal Changes in the Composition of the Pecan during Fruit Development 97–121
Smut, reactions of corn to, experiments 47–56
SNELL, M. E., and BEAUMONT, A. B.: The Effect of Magnesium Deficiency on Crop Plants 553–562
Sodium selenate, toxicity to wheat, experiments 415–424

Soil— Page
clay, heavy, soil-moisture extraction in, relation to pear root concentration. W. W. Aldrich, R. A. Work, and M. R. Lewis 975–988
moisture—
effect on freezing of winter wheat, experiments 629–630
extraction in heavy clay soil, relation to pear root concentration. W. W Aldrich, R. A. Work, and M. R. Lewis 975–988
sulphur content, effect on absorption of selenium by plants 417–420, 425
types, effect on yield and composition of oats and vetch. G. O. Baker and S. C. Vandecaveye 967–974
Soils—
magnesium-deficient, relation to chlorosis in plants 553–562
selenium content, absorption by plants, factors affecting. Annie M. Hurd-Karrer 413–427
virgin longleaf pine, effect of annual grass fires on organic matter and other constituents. S. W. Greene 809–822
Sorgo—
absorption of selenium from soils, factors affecting 413–415
× sugarcane hybrids, morphological characters, comparative studies. B. A. Bourne 539–552
Southern States, logs and lumber in, fungi including five new species causing stain in. Ross W. Davidson 789–807
Southwest, ponderosa pine, yield and mortality, factors influencing. B. R. Loxen 777–787
Spinach, chlorosis caused by magnesium deficiency, study 555, 557, 558–559
STONE, B. M., and SIMPSON, D. M.: Viability of Cottonseed as Affected by Field Conditions 435–447
Storage rot fungi, infection and decay of sweetpotatoes, factors affecting. J. I. Lauritzen 285–329
Strawberries—
chemical—
composition and resistance to puncture, analyses 650–659
constituents, relation of changes to puncture resistance, studies 664–679
development, physiological changes, tests 660–664
preserving, requirements 688–692
ripening, chemical changes 663–664
Strawberry—
development and ripening, physiological study. Charles W. Culpepper, Joseph S Caldwell, and Hubert H. Moon 645–696
utilization, relation of chemical and physical characters to 687–692
varieties, physiological studies 681–687
STRITAR, J.; OSBURN, O. L.; and WERKMAN, C. H.: The Thermophilic Fermentation of Beet Pulp 165–172
STROMAN, G. N.: Procedure for Rapid Calculation of Multiple Correlation Coefficients 59–69
Strophostyles rust, comparison with bean and cowpea rusts 742
Sturmia scutellata, parasitization by *Brachymeria compsilurae* 517–518, 520
Sudan grass, growth, relation to nutrient value of phosphorus on, greenhouse experiments 841–846

Page

Sugar-beet leaf hopper, agent in spread of curly top virus, study 212–213, 219–222

Sugar beets—
- bacterial pocket rot, causal organism, studies 933–951
- sources of curly top virus, studies 212–217
- stand variations, effect on yield and quality under irrigation. H. E. Brewbaker and G. W. Deming 195–210

Sugarcane × sorgo hybrids, morphological characters, comparative study. B. A. Bourne 539–552

Sulphur content of soil, effect on absorption of selenium by plants 417–420, 425

Surface rot of sweetpotatoes, factors affecting 301–303, 324–327

Sweetpotato, five varieties of, vitamin A content. F. L. MacLeod, M. R. Armstrong, M. E. Heap, and L. A. Tolbert 181–187

Sweetpotatoes—
- infection and decay by certain storage rot fungi, factors affecting. J. I. Lauritzen 285–329
- storage—
  - effect on vitamin A content, experiments 181–186
  - rots, factors affecting 285–329
- vitamin A content of five varieties. F. L. MacLeod, M. R. Armstrong, M. E. Heap, and L. A. Tolbert 181–187

Swift, R. W.; Braman, Winfred W.; Black, Alex; Kahlenberg, O. J.; Voris, LeRoy; and Forbes, E. B.: The Utilization of Energy-Producing Nutriment and Protein in White and Yellow Corn and in Diets Deficient in Vitamins A, D, and G 1–37

Swine—
- erysipelas, serological studies with particular reference to agglutination. H. W. Schoening and G. T. Creech 71–79
- feed consumption, effect of ergot upon, experiments 43–44
- feeding rye containing ergot, experiments. D. W. Johnson and L. S. Palmer 39–45
- growth, effect of ergot upon, experiments 42–43
- infection with *Erysipelothrix rhusiopathiae*, experiments 71–74

Temperature—
- effect on—
  - activity of curly top virus, experiments 226–229
  - development of uredial stage of *Puccinia graminis*. Leonard W. Melander 861–880
  - infection and decay of sweetpotatoes, studies 285–327
  - Mexican bean beetle in Mexico 994–995
  - productivity of camphor scale, study 246–247
  - settling of camphor scale, study 260–262
  - of artificial drying, effects on digestibility and availability of nutrients in pasture herbage. R. E. Hodgson, J. C. Knott, R. R. Graves, and H. K. Murer 149–164
  - winter, effect on bud sports of Montmorency cherry 465–469
- Temperatures, low, injury to flower buds of Montmorency cherry. V. R. Gardner 563–572

Page

Teosinte, perennial and maize, tetraploid hybrid of. G. N. Collins and A. E. Longley 123–133

Tetraploid hybrid of maize and perennial teosinte. G. N. Collins and A. E. Longley 123–133

Tetraploidy, study in maize hybrids 123–133, 596–603

Thor, Clifford J. B., and Smith Charles L.: A Physiological Study of Seasonal Changes in the Composition of the Pecan during Fruit Development 97–121

Tidmore, J. W.; Jacob, K. D.; Bartholomew, R. P.; Brown, B. E.; Pierre, W. H.; and Reid, F. R.: Nutrient Value of the Phosphorus in Calcined Phosphate as Determined by Growth of Plants in Greenhouse Experiments 837–848

Timothy—
- stem rust, development, studies 861–879
- yield and chemical composition 712–715

Titus, Harry W.; DeVaney, Grace M.; and Nestler, Ralph B.: Vitamin A Content of Eggs Produced by Chickens Fed Viosterol and Various Percentages of Cod-Liver Oil 853–860

Tobacco, chlorosis caused by magnesium deficiency, study 558, 562

Tolbert, L. A.; MacLeod, F. L.; Armstrong, M. R.; and Heap, M. E.: The Vitamin A Content of Five Varieties of Sweetpotato 181–187

Toxicity, rotenone, new criterion for comparison with respect to concentration and time. W. A. Gersdorff 881–891

Tribolium confusum Duval, vitamin D needs, and phosphorus content and requirements, study. J. W. Nelson and L. S. Palmer 849–852

Tricholaena rosea, decomposition rate in Norfolk sand, experiments 717–729

Trichopria hirticollis, parasitism on blowfly pupae 481, 491

Trichosporium symbioticum, n. sp.—
- association with *Scolytus ventralis*. Ernest Wright 525–538
- cultural characteristics and morphology 527–529
- moisture relationship tests 536–537
- occurrence and dissemination by *Scolytus ventralis* 529–532
- pathogenicity tests 532–536
- technical description 527

Tullis, E. C.: Histological Studies of Rice Leaves Infected with *Helminthosporium oryzae* 81–90

Turnips, chlorosis caused by magnesium deficiency, study 554, 555, 558

Uromyces phaseoli—
- *typica*—
  - cause of bean rust, studies. L. L. Harter, C. F. Andrus, and W. J. Zaumeyer 737–759
  - development, environmental factors 742–745
  - spore forms, studies 738–742
  - *See also* Bean rust.
- *vignae*, comparison with *Uromyces phaseoli typica* 741–742

Ustilago zeae collections, field reaction of varieties and selfed lines of corn to. J. J. Christensen and I. J. Johnson 47–57

VANDECAVEYE, S. C., and BAKER, G. O.: Effect of Fertilizers, Soil Type, and Certain Climatic Factors on the Yield and Composition of Oats and Vetch ... Page 961–974

Vegetables, chlorosis caused by magnesium deficiency, studies ... 554–561

Vein spot disease of pecan foliage, fungus causing, perfect stage of, *Gnomonia nerviseda*. J. R. Cole ... 91–96

Vetch, composition and yields, effect of fertilizers, soil type, and certain climatic factors on. G. O. Baker and S. C. Vandecaveye ... 961–974

Vigna sinensis. *See* Cowpea.

Viosterol, feeding to chickens, effect on vitamin A content of eggs. Grace M. DeVaney, Harry W. Titus, and Ralph B. Nestler ... 853–860

Vitamin—
A—
content of—
eggs, production by feeding chickens viosterol and percentages of cod-liver oil. Grace M. DeVaney, Harry W. Titus, and Ralph B. Nestler ... 853–860
five varieties of sweetpotato. F. L. MacLeod, M. R. Armstrong, M. E. Heap, and L. A. Tolbert ... 181–187
deficiency in corn, experiments with rats ... 2–6, 13–1[illegible]
D—
deficiency in corn, experiments with rats ... 19–26
requirements of *Tribolium confusum*, study ... 849–852
G, deficiency in corn, experiments with rats ... 26–

VORIS, LEROY; BRAMAN, WINFRED W.; BLACK, ALEX; KAHLENBERG, O. J.; SWIFT, R. W.; and FORBES, E. B.: The Utilization of Energy-Producing Nutriment and Protein in White and Yellow Corn and in Diets Deficient in Vitamins A, D, and G ... 1–37

WALKER, J. C., and ANDERSON, M. E.: Histological Studies of Wisconsin Hollander and Wisconsin Ballhead Cabbage in Relation to Resistance to Yellows ... 823–836

Weather—
data at Acclimatization Field Station, 1931–33 ... 436–437
factor in germination of cottonseed ... 435–446

WEBBER, IRMA E.: Histological Characteristics of Plants Grown in Toxic Concentrations of Boron ... 189–194

WERKMAN, C. H.; OSBURN, O. L.; and STRITAR, J.: The Thermophilic Fermentation of Beet Pulp ... 165–172

Wheat—
freezing tests, artificial, technic ... 627–632
grain yield among 1,500 nursery plots, variation and correlation. Gustav A. Wiebe ... 331–357
jointworm—
control, recommendation ... 367–368, 384
gall—
microchemical tests and tissue zones, studies ... 377–379

Wheat—Continued.
jointworm—Continued.
gall—Continued.
morphology and biology. W. J. Phillips and F. F. Dicke ... Page 359–386
seasonal history ... 369–377
stimulus, investigations ... 379–383
See also *Harmolita tritici*.
nursery plots, 1,500, variation and correlation in grain yield. Gustav A. Wiebe ... 331–357
plants, absorption of selenium from soils, factors affecting ... 413–426
seedlings, age, effect on cold resistance ... 629
stem—
gall. *See* Wheat jointworm gall.
rust, development, studies ... 861–879
winter—
cold-resistance inheritance, with preliminary studies on technic of artificial freezing tests. W. W. Worzella ... 625–635
hardening against freezing, experiments ... 628

WIEBE, GUSTAV A.: Variation and Correlation in Grain Yield among 1,500 Wheat Nursery Plots ... 331–357

WILMOTH, J. H.; ACKERT, J. E.; EISENBRANDT, L. L.; GLADING, B.; and PRATT, I.: Comparative Resistance of Five Breeds of Chickens to the Nematode *Ascaridia lineata* (Schneider) ... 607–624

WITHROW, ROBERT B.: A Photoelectric Device for the Rapid Measurement of Leaf Area ... 637–643

Wood-staining—
fungi occurring in Southern States, study ... 789–806
fungus, *Trichosporium symbioticum*, n. sp., associated with *Scolytus ventralis*. Ernest Wright ... 525–538

WORK, R. A.; ALDRICH, W. W.; and LEWIS, M. R.: Pear Root Concentration in Relation to Soil-Moisture Extraction in Heavy Clay Soil ... 975–988

WORZELLA, W. W.: Inheritance of Cold Resistance in Winter Wheat, with Preliminary Studies on the Technic of Artificial Freezing Tests ... 625–635

WRIGHT, ERNEST: *Trichosporium symbioticum*, N. Sp., Wood-Staining Fungus Associated with *Scolytus ventralis* ... 525–538

Xylosema—
armata, distribution and habits ... 487–488
sp., distribution and habits ... 488–489

Yellows in cabbage, resistance of Wisconsin Hollander and Wisconsin Ballhead varieties, histological studies. M. E. Anderson and J. C. Walker ... 823–836

ZAUMEYER, W. J.; HARTER, L. L.; and ANDRUS, C. F.: Studies on Bean Rust Caused by *Uromyces phaseoli typica* ... 737–759

Zea mays. *See* Corn; Maize.